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FOLIC ACID IN VEGETABLES AND CERTAIN OTHER PLANT MATERIALS[1]

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Although the folic acid content of several foods has been reported by a number of investigators, Cheldelin and Williams (1942); Cheldelin, Woods, and Williams (1943); Luckey *et al.* (1945); Ives *et al.* (1946); Schweigert, Pollard, and Elvehjem (1946); Olson, Burris, and Elvehjem (1947), in most studies takadiastase has been used in the enzymatic treatment of samples for the liberation of the vitamin prior to microbiological assay. Olson *et al.* (1948A) have found a hog kidney conjugase, prepared as described by Bird *et al.* (1945), much superior to takadiastase in liberating folic acid from plant samples. This paper reports the results of some investigations of the folic acid content of plant materials in which hog kidney conjugase has been used to liberate the vitamin prior to microbiological assay.

METHODS

Plant samples were prepared for assay by grinding them twice through a Nixtamal mill. When the samples were small, a mortar and pestle were used for the grinding. After mixing the ground samples well, 0.5-gram portions were suspended in five ml. of one per cent sodium acetate buffer, pH 4.5, and heated for three minutes in a boiling water bath. After cooling heated mixture, two ml. of a water extract of hog kidney, prepared and stored as described by Bird *et al.* (1945), were added. The mixture was incubated under toluene at 37°C.(99°F.) for 24 hours. It was then heated for two minutes in a boiling water bath to coagulate proteins, cooled, and neutralized to pH 6.8 to 7.0 with two N NaOH. It was finally made up to 100 ml. and filtered.

Samples of canned vegetables were ground for five minutes in a Waring blendor and one-gram portions prepared for assay as described above.

Dry samples were finely ground in a Wiley mill and then well mixed; 100-mg. portions were suspended in five ml. of the sodium acetate buffer and autoclaved for five minutes at 15 pounds' pressure. After cooling, the samples were prepared for assay by the same method used for the fresh plant samples, except they were filtered with gentle suction through a a thin pad of Supercel.

Aliquots of the filtrates prepared were made up to an appropriate volume and assayed microbiologically for folic acid. Unless otherwise indicated, the assays were made turbidimetrically, using both *Streptococcus faecalis* with the method of Luckey, Briggs, and Elvehjem (1944) (medium

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modified by adding salts B) and *Lactobacillus casei* with the method of Roberts and Snell (1946) (modified by increasing the time of incubation to 36 hours). Synthetic pyteroylglutamic acid (Lederle Laboratories, Inc.) was used as a standard in both assays. Blank determinations were made on the enzyme preparation in all cases and have been subtracted in all results given here.

Moisture determinations were made by drying the samples at from 75 to 80°C. (167 to 176°F.) for 48 hours.

TABLE 1

Distribution of Folic Acid in the Tobacco Plant

Size of plant	Part of plant	Folic acid content (γ/gm.)			
		Wet basis		Dry basis	
		S. faecalis	*L. casei*	*S. faecalis*	*L. casei*
15 inches	Growing tip of stem and very small leaves	0.97	0.92	11.6	11.0
	Upper large leaves	0.80	0.82	11.1	11.4
	Lower leaves	0.15	0.13	2.6	2.1
	Stem	0.11	0.16	1.5	2.2
	Leaf midribs (from leaves at central part of plant)	0.05	0.04	1.4	1.1
	Central leaves minus midrib	0.63	0.63	8.5	8.5
	Roots	0.51	0.48	3.1	2.9
24 inches	Upper leaves	2.60	2.20	.....	.....
	Lower leaves	0.13	0.15	.....	.....

TABLE 2

Distribution of Folic Acid in the Bean Plant

Stage of growth	Part of plant	Folic acid content (γ/gm.)			
		Wet basis		Dry basis	
		S. faecalis	*L. casei*	*S. faecalis*	*L. casei*
Maturing	Upper large leaves	1.35	1.30	9.5	9.1
	Lower leaves	1.13	0.96	8.6	7.5
	Small beans	0.93	0.79	10.2	8.7
	Large beans	0.90	0.81	6.9	6.2
	Seeds (older beans)	1.35	1.17	5.7	4.9
	Pods (older beans)	0.50	0.47	4.2	4.0
Flowering	Upper small leaves	2.40	2.42	....	....
	Upper large leaves	1.62	1.35	....	....
	Lower leaves	0.76	0.73	....	....
	Stems and petioles	0.26	0.24	....	....
	Flowers	0.68	0.69	....	....

EXPERIMENTAL RESULTS

Distribution of Folic Acid in Plants: Results of studies on the distribution of folic acid in the bean and tobacco plant are presented here because they demonstrate that reasonable care must be exercised in the sampling of green plants for the vitamin assay. Single tobacco plants were divided (indicated Table 1) and the various parts ground with a mortar and pestle for analysis. The same was done with bean plants (Table 2)

TABLE 3

Folic Acid Content of the Edible Portions of Fresh Vegetables

Vegetable	Micro-organisms used for assay [1]	No. of samples assayed	Folic acid content (γ/gm.)			
			Wet basis		Dry basis	
			Range	Average	Range	Average
Spinach	S.F.	3	2.6 -3.0	2.8	19.0 -27.0	22.3
	L.C.	2	2.2 -2.3	2.25	16.0 -22.0	19.0
New Zealand spinach	S.F.	1	.....	1.7	.....	16.0
	L.C.	1	.....	1.6	.....	15.0
Swiss chard	S.F.	5	.44-1.25	.70	3.2 -10.0	6.2
	L.C.	6	.40-1.25	.62	3.2 -10.0	5.3
Leaf lettuce	S.F.	4	.58-1.40	.84	9.3 -12.3	10.3
	L.C.	4	.47-1.10	.69	6.8 - 9.7	8.5
Endive	S.F.	4	.66- .89	.75	6.3 - 8.0	7.9
	L.C.	4	.50- .75	.62	5.6 - 7.5	6.5
Kale	S.F.	1	.....	.92	.....	5.8
	L.C.	1	.....	1.00	.....	6.5
Parsley	S.F.	1	.....	1.70	.....	12.0
	L.C.	1	.....	1.70	.....	12.0
Asparagus	S.F.	1	.....	1.24	.....	12.0
	L.C.	1	.....	1.18	.....	11.7
Broccoli	S.F.	1	.....	.90	.....	.....
	L.C.	1	.....	1.10	.....	.....
Beet greens [2]	L.C.	1	.....	.25	.....	2.3
Beets [2]	L.C.	1	.....	.42	.....	2.4
Kohlrabi	S.F.	2	.48- .56	.52	4.7 - 6.4	5.55
	L.C.	2	.43- .56	.50	4.9 - 5.5	5.2
Cauliflower	S.F.	1	.....	.44	.....	.....
Green beans	S.F.	4	.54- .90	.71	4.8 - 8.2	6.2
	L.C.	4	.51- .81	.71	4.8 - 7.7	6.2
Wax beans	S.F.	2	.18- .35	.27	1.6 - 3.8	2.7
	L.C.	2	.13- .33	.23	1.2 - 3.6	2.4
Peas	S.F.	2	.19- .26	.23	.97- 1.3	1.14
	L.C.	2	.20- .23	.22	1.0 - 1.1	1.05
Radishes	S.F.	2	.12- .13	.13	2.1 - 2.3	2.2
	L.C.	2	.10- .12	.11	1.8 - 2.2	2.0
Tomatoes	S.F.	1	.....	.14	.....	.....
	L.C.	1	.....	.12	.....	.....

[1] S.F. = *S. faecalis*; L.C. = *L. casei*. [2] Acidimetric assay [Method of Teply and Elvehjem (1945) modified to omit the norit-treated peptone from the medium].

except that three plants were combined for sampling. Differences in the folic acid content of the various parts of the plants were very large. The more rapidly growing tissues apparently contain the higher concentrations of the vitamin. It is reasonable to expect that similar variations will occur in other plants; this necessitates care in sampling vegetables for folic acid assays.

Folic Acid Content of Some Fresh and Canned Vegetables: Folic acid assays of several fresh garden vegetables are given (Table 3). In a few cases, analyses of greenhouse-grown vegetables are included in the results. All samples were prepared for assay within two hours after harvesting. Values obtained in this study are, in general, higher than those obtained by Olson, Burris, and Elvehjem (1947). However, with few exceptions the comparative folic acid contents of the several vegetables in this and earlier investigations are very similar. Many of the vegetables, especially the green, leafy type, are good sources of the vitamin. Here, contrary to our previous findings, leaf lettuce has been found to compare favorably with several of the other leafy vegetables.

Large variations in the folic acid content of certain of the vegetables (chard and lettuce especially) indicate that such factors as stage of growth or state of nutrition of the plants may affect their vitamin content. The higher values obtained with *S. faecalis* than with *L. casei* cannot be explained. It may be that, in addition to pteroylglutamic acid and pteroylglutamic acid conjugate, pteroic acid, or the SLR factor of Keresztesy, Rickes, and Stokes (1943), is present in plant tissues, but direct evidence for this has not been obtained.

TABLE 4

Folic Acid Content of Canned Vegetables

Vegetable[1]	Folic acid content (γ/gm.)			
	Wet basis		Dry basis	
	S. faecalis	*L. casei*	*S. faecalis*	*L. casei*
Canned spinach 1	0.93	0.84	13.40	12.10
Canned spinach 1	0.92	0.83	12.40	11.20
Canned spinach 2	0.43	0.41	6.20	5.90
Canned spinach 3	0.12	0.12	1.90	1.90
Canned spinach 4	0.51	.....	5.35	.....
Canned spinach 5	0.93	.....	11.10	.....
Canned asparagus	0.22	0.21	3.53	3.37
Canned peas	0.12	0.10	0.81	0.64
Canned green beans	0.11	0.07	1.90	1.27
Canned wax beans	0.08	0.06	1.82	1.36

[1] Numerals represent various brands.

The folic acid content of a few canned vegetables is given (Table 4). After observing the large variations in the different samples of canned spinach, it was felt that unless large numbers of samples, including several brands, were assayed, reliable average values could not be obtained. Since large numbers of samples were not available, no further studies of canned goods were made. Variations observed with the canned spinach indicate a need for an investigation of the effect of the canning process on the folic acid content of canned goods. Results obtained in these studies are

in general higher than those obtained by Ives *et al.* (1946), who used

TABLE 5

Effect of Various Storage Methods on the Folic Acid Content of Fresh Vegetables

Vegetable	Micro-organism [1]	Folic acid content of vegetables under various storage conditions (γ/gm., dry basis)						
		Fresh	Room temperature			Refrigerator		Crushed ice
			1 day	2 days	3 days	1 week	2 weeks	1 week	2 weeks	2 weeks
Parsley	S. F.	12.00	.....	.....	7.00	.....	10.00	.....	14.00
	L. C.	12.00	.....	.....	6.20	.....	8.30	.....	14.00
Green beans	S. F.	5.00	3.10	.....	2.20	2.80	.....	3.40	.....
	L. C.	4.80	2.90	.....	2.50	3.70	.....	4.10	.....
Peas	S. F.	0.97	0.88	.....	0.56	0.89	0.89	1.12	1.08
	L. C.	1.02	0.93	.....	0.51	0.70	1.08	1.12	1.08
Asparagus	S. F.	12.00	9.30	5.60	3.20	8.80	8.40	9.90	14.00
	L. C.	12.00	7.50	5.60	2.60	9.10	7.20	9.60	11.00
Endive	S. F.	6.30	5.20	3.80	1.80	4.90	6.50	6.70	7.60
	L. C.	7.10	6.60	4.90	2.60	4.70	5.80	6.30	6.10
Spinach	S. F.	27.00	20.00	20.00	16.00	20.00	27.00	22.00	31.00
	L. C.	22.00	19.00	17.00	14.00	21.00	18.00	23.00	21.00
Swiss chard	S. F.	7.30	7.40	8.30	6.30	8.40	7.00	9.50	9.70
	L. C.	5.70	6.00	8.00	4.60	6.10	6.40	7.70	10.00
Swiss chard	S. F.	3.90	3.20	2.80	.....	4.80	3.20	5.00	3.50
	L. C.	3.60	3.20	2.20	.....	4.20	3.30	4.30	3.50
Swiss chard	S. F.	10.00	.....	.....	4.80	.....	5.40	.....	12.00
	L. C.	10.00	.....	.....	7.40	.....	6.50	.....	13.00
Leaf lettuce	S. F.	10.00	10.00	9.00	.....	15.00	.....	20.00	.....
	L. C.	9.00	10.00	8.00	.....	12.00	.....	15.00	.....
Leaf lettuce	S. F.	9.00	11.00	9.00	.....	11.00	.....	13.00	.....
	L. C.	9.00	10.00	10.00	.....	10.00	.....	11.00	.....

[1] Microorganism used for microbiological assay: S.F. = *S. faecalis*; L.C. = *L. casei*.

TABLE 6

Folic Acid Content of Feedstuffs

Sample	Folic acid content (γ/gm.)					
	S. faecalis			*L. casei*		
	No. of samples	Range	Average	No. of samples	Range	Average
Ground soybeans	1	.....	5.30	1	.....	3.60
Ground barley	5	.39-.63	0.49	5	.29- .51	0.41
Ground yellow corn	7	.22-.44	0.29	6	.21- .29	0.23
Ground oats	7	.28-.55	0.40	7	.26- .54	0.34
Ground rye	5	.65-.94	0.73	5	.41- .57	0.45
Ground wheat	6	.53-.68	0.62	6	.47- .60	0.55
Wheat bran	1	.....	2.35	3	1.53-1.58	1.55
Wheat middlings	1	.....	1.76	2	1.28-1.48	1.38
Wheat germ	1	.....	3.30	1	.....	2.80
Soybean oil meal	....	No analysis	.....	1	.....	3.80
Linseed oil meal	....	No analysis	.....	1	.....	1.00
Alfalfa hay	1	.....	3.00	1	.....	2.40
Timothy hay	1	.....	3.00	1	.....	2.30

cereals are a fairly good source of folic acid, but on a dry weight basis

in general higher than those obtained by Ives *et al.* (1946), who used takadiastase to liberate the vitamin.

Effect of Various Methods of Storage on the Folic Acid Content of Fresh Vegetables: Preliminary studies of the effect of various storage conditions on the folic acid content of vegetables reported by Olson *et al.* (1947) indicated that losses which occurred at room temperature could be prevented for periods of up to two weeks by storing the vegetables in crushed ice or in the refrigerator. However, in most cases the folic acid content of the vegetables increased during the storage period at low temperature, and it was concluded that the increase was the result of the liberation of folic acid from its conjugate during storage. Results reported here are a continuation of the preliminary work, differing only in the use of hog kidney conjugase instead of takadiastase for liberation of folic acid.

TABLE 7

Folic Acid Content of Breakfast Foods

Basic constituent	No. of samples analyzed	Folic acid content (γ/gm.)			
		S. faecalis		*L. casei*	
		Range	Average	Range	Average
Wheat bran [1]	5	.51-1.07	.81	.51-.96	.77
Whole wheat	18	.21- .50	.39	.21-.59	.40
Hulled oats	3	.32- .59	.50	.30-.46	.40
Corn	5	.06- .12	.09	.07-.13	.09
Rice	3	.06- .12	.09	.08-.11	.10

[1] Probably contain about an equal amount of whole wheat.

The vegetables were obtained fresh from the garden or greenhouse. They were immediately divided into several groups for storage. To insure that all samples were of equivalent maturity, the vegetables were first divided into several size groups and then the size groups divided equally into several samples for storage. The samples were placed in wax bags and then stored at room temperature, in the refrigerator at 4 to 5°C.(39 to 41°F.), or in crushed ice. The crushed ice was placed in and around the bag and replenished as needed. One sample was prepared immediately for assay; other samples were prepared for assay as indicated (Table 5).

As in our earlier work, storage of vegetables at room temperature resulted in large losses of folic acid, whereas refrigeration or storage in crushed ice was effective in preventing losses. Storage in the crushed ice was somewhat more effective than refrigeration in this respect, and for the preservation of the fresh appearance of the vegetables, crushed ice was much superior to refrigeration. Again, increases in folic acid were found in several instances when the vegetables were stored in the cold. Possible explanations for this will be discussed later in this paper.

Folic Acid Content of Certain Grains and Breakfast Cereals: The folic acid content of cereal grains is low compared to that of certain other feedstuffs (Table 6). Values obtained here are in fair agreement with those obtained by Lillie and Briggs (1947) who used the chick assay. As might be expected, breakfast foods prepared from the cereals contain about the same amount of the vitamin as do the cereals (Table 7). The bran

cereals are a fairly good source of folic acid, but on a dry weight basis they contain far less folic acid than do the green leafy vegetables. However, if cooking vegetables results in large losses of folic acid, as the work of Cheldelin *et al.* (1943) indicates, the bran breakfast cereals may be a comparatively good dietary source of the vitamin, since they are eaten without cooking.

DISCUSSION

The method of assay used in the studies discussed here apparently measures all pteroylglutamic acid and PGA conjugate present in plant samples, Olson *et al.* (1948A). Results with *S. faecalis* should also include pteroic acid and SLR factor if they are present. However, it has been found, Olson *et al.* (1948B), that rat livers contain a precursor of folic acid which cannot be determined by the method used here. It is possible that a similar substance occurs in plants, which might explain results obtained in the storage studies (especially with lettuce). For instance, if a "bound" form of folic acid, not measured by the method used here, is present in plant materials, it may be released slowly by enzymes in the plant tissues at the low temperatures. Its release at higher temperatures (room temperature) may be masked by a rapid destruction of folic acid. Storage in the refrigerator or in crushed ice markedly reduces the rate of destruction of the vitamin, and the apparent release of the "bound" form becomes noticeable. However, experimental proof of the occurrence of such "bound" forms of folic acid in plant materials is still lacking.

SUMMARY

A hog kidney conjugase has been used to liberate bound folic acid in plant materials. The folic acid contents of several samples of fresh vegetables, canned vegetables, grains, and breakfast cereals are reported. Large variations in the folic acid content of various parts of plants were observed. The destruction of folic acid which occurs when fresh vegetables are stored at room temperature is largely prevented by storage in a refrigerator (4 to 5°C.) or even more completely by storage in crushed ice. Data accumulated indicate that "bound" forms of folic acid other than folic acid conjugate may occur in plant tissues.

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ASCORBIC ACID LOSSES AND DARKENING ON STORAGE AT 49°C.(120°F.) OF SYNTHETIC MIXTURES ANALOGOUS TO ORANGE JUICE [1]

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Orange juice on storage at room temperature deteriorates in flavor much more rapidly than other juices such as apple, pineapple, grape, and tomato. Deterioration in flavor is generally accompanied by other changes, in particular, darkening in the aqueous phase and losses of ascorbic acid. The work reported here was carried out in the hope of obtaining some information relative to the reactants involved. A number of synthetic mixtures containing various combinations of ascorbic acid, amino acids, sugars, citric acid, and potassium citrate were prepared, canned, stored at 49°C.(120°F.), and examined periodically.

EXPERIMENTAL PROCEDURE

General Plan of Work: The storage studies were carried out at 49°C. in order to save time, since it was anticipated that results with the first series of packs (Table 1, Nos. 1 to 6) would lead to the preparation of others. Earlier work by the author, Curl (1947), on concentrated orange juice showed that the changes occurring at 27 and 49°C.(80 and 120°F.) were similar, but that the rate of change was about 20 times as fast at the higher temperature. When stored at 80°F., single-strength orange juice lost, in twelve months, only 30 per cent of its original ascorbic acid.

The object in Pack 1 was to study the rate of loss of ascorbic acid from a solution containing in addition only citric acid, potassium citrate, and sodium benzoate, none of which would be expected to act as an oxidizing or reducing agent. It appeared possible that ascorbic acid might undergo a disproportionation or other decomposition involving no oxidizing agent.

Effects on ascorbic acid loss of (1) sugars, (2) amino acids, and (3) amino acids plus sugars, were studied in Packs 2, 3, and 4, respectively. Packs 5 and 6 were the same as Packs 4 and 2, respectively, but with no ascorbic acid, the object here being to study the degree of darkening.

The quantity of ascorbic acid used in Packs 1 to 4 and also 7 to 11 corresponds to that in an orange juice of fairly high ascorbic acid content (50 mg./100 ml.). Mixture of sugars used (five per cent sucrose, 2.5 per cent anhydrous dextrose, and 2.5 per cent levulose) was similar to that which probably occurs in orange juice, Curl and Veldhuis (1947). For amino acids, a mixture of 0.2 per cent each of *dl*-alanine, *l*-asparagine, and *d*-arginine (as the hydrochloride) was used. Asparagine and arginine,

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together with stachydrine, choline, and aspartic acid, were found by Nelson, Mottern, and Eddy (1933) to be present in orange juice. Alanine was used because it is a typical monoamino monobasic acid. According to Chatfield and Adams (1940), orange juice contains 0.6 per cent of crude protein; hence this quantity of the mixed amino acids was used in Packs 3, 4, and 5.

On completion of storage studies on Packs 1 to 6, a second group of packs was prepared. Packs 7 to 9 were similar to No. 2 except that each contained only one sugar—sucrose, dextrose, or levulose. Pack 10 was similar to Nos. 7 to 9 except that sorbitol, a hexahydric alcohol, was used instead of a sugar.

In Packs 11 to 13 the object was to note the effect of the concentration of ascorbic acid on the rate of ascorbic acid loss in the absence of sugars or amino acids. Pack 11 was almost identical with Pack 1.

Each pack in both plain tin and enamel-lined cans was examined for ascorbic acid content and color density after storage for 30 and 60 days at 49°C. The initial analysis was made on the day following the preparation of the samples, the cans having been allowed to stand overnight at room temperature.

Preparation of Samples: Composition of the 13 mixtures is given (Table 1). Each contained one per cent of citric acid and 0.7 per cent of potassium citrate (both as hydrates) to buffer the solutions to a pH of about 3.7 (Table 1), which falls within the normal range of pH of orange juice. These compounds are part of the buffer system of orange juice. Sodium benzoate (0.1 per cent) was added to each mixture as a preservative. The mixtures were filled at room temperature into 211 by 210 citrus-enamel and plain tin cans, and closed under 20 inches of vacuum. The solutions were not pasteurized, since no enzymes were present and contamination with microorganisms was probably small. One or two cans of each mixture were reserved for the initial examination and the remainder stored at 49°C.

Methods of Analysis: Ascorbic acid was determined as soon as possible after the can was opened by titration with 2,6-dichlorophenolindophenol, one-half per cent oxalic acid solution being used as a stabilizing agent.

Color density was measured by diluting a 25-ml. aliquot of the sample to 50 ml. with acetone and determining the percentage transmission with an Evelyn photoelectric colorimeter (Filter No. 420). This is the same method as used with orange juice, Curl (1947). The color density is equal to two minus the log of the percentage transmission.

The pH was measured in the initial samples only. The pH values ranged from 3.70 to 3.80 except for Pack 13 which contained 500 mg./100 ml. of ascorbic acid and had a pH of 3.64 (Table 1).

RESULTS AND DISCUSSION

Ascorbic acid analyses of the initial and storage samples are given (Table 2). Increases in color density of Packs 1 to 6 are given (Table 3).

Ascorbic Acid Retention: There was a rapid loss in ascorbic acid of from five to seven mg. per 100 ml. during the first day in enamel cans, as

TABLE 1

Composition and pH of Synthetic Mixtures Analogous to Orange Juice[1]

Pack No.	Ascorbic acid (approximate)	Other constituents	pH
	mg./100 ml.		
1	50	.....	3.72
2	50	5% sucrose, 2.5% dextrose, 2.5% levulose	3.72
3	50	0.2% each *dl*-alanine, *l*-asparagine, *d*-arginine	3.75
4	50	Sugars as in No. 2, amino acids as in No. 3	3.80
5	0	Sugars as in No. 2, amino acids as in No. 3	3.71
6	0	Sugars as in No. 2	3.73
7	50	10% sucrose	3.75
8	50	10% dextrose	3.70
9	50	10% levulose	3.71
10	50	10% sorbitol	3.75
11	50	.....	3.78
12	150	.....	3.72
13	500	.....	3.64

[1] All mixtures contained one per cent citric acid (hydrate), 0.7 per cent potassium citrate (hydrate), and 0.1 per cent sodium benzoate.

TABLE 2

Initial Ascorbic Acid Contents of Synthetic Mixtures Analogous to Orange Juice and Percentage Loss After Storage at 49°C.(120°F.)

Pack No.	Plain tin cans			Citrus-enamel cans		
	Initial contents	Pct. loss		Initial contents	Pct. loss	
		30 days	60 days		30 days	60 days
	mg./100 ml.			*mg./100 ml.*		
1	49.6[1]	18	32	44.6	22	37
2	44.1[1]	29	54	39.1	33	58
3	45.9[1]	18	33	40.9	23	37
4	49.6[1]	31	59	44.6	35	64
7	49.8	26	67	44.4	45	77
8	48.7	25	50	44.1	40	61
9	49.6	37	88	44.6	61	89
10	46.2	19	29	44.0	28	38
11	50.3	19	27	44.3	29	43
12	149	25	38	142	29	45
13	496	37	54	490	31	48
Orange juice	.....	....	....	27.1	44	79

[1] Estimated.

TABLE 3

Color Density Increases in Synthetic Mixtures Analogous to Orange Juice After Storage at 49°C.

Pack No.	Plain tin cans		Citrus-enamel cans	
	30 days	60 days	30 days	60 days
1	.006	—.008	.019	.013
2	—.001	.020	.052	.098
3	.007	.004	.027	.032
4	.019	.159	.130	.478
5	.003	.078	.092	.299
6	—.008	.008	.009	.020
Orange juice	.....	.....	.344	.870

shown in Packs 7 to 13. This loss was not evident in the plain tin cans, where the results closely approximated the amounts added. This difference is probably due to the residual oxygen in the can reacting with the tin in one case and with the ascorbic acid in the other. Initial ascorbic acid determinations were not made on the samples of Packs 1 to 4 in plain tin cans, but the values were estimated on the basis of experience gained in the remainder of packs.

In addition to the rapid initial loss of ascorbic acid from samples in enamel cans, such samples continued to lose ascorbic acid more rapidly in storage than did those in plain tin cans, except in Pack 13, in which the amount of ascorbic acid added was exceptionally high.

Considerable losses of ascorbic acid occurred in the absence of sugars and amino acids (Pack 1), indicating that ascorbic acid may disappear in the absence of any oxidizing agent. Addition of amino acids (Pack 3) did not affect the loss of ascorbic acid, indicating that amino acids are without effect in the absence of sugars. The addition of sugars (Pack 2) made the losses about 50 per cent greater, indicating that a reaction of ascorbic acid with the sugars, or with a conversion product of the sugars, is probably responsible for a considerable part of the losses. Addition of both amino acids and sugars (Pack 4) resulted in a little greater loss of this vitamin. This indicates that amino acids may have some effect in the presence of sugars.

Addition of levulose (Pack 9) resulted in considerably greater loss of ascorbic acid than did the addition of dextrose (Pack 8). Addition of sucrose (Pack 7) showed the same effect as added dextrose after one month of storage, but after two months the loss of ascorbic acid was greater in the pack containing sucrose. This is probably due to the increased content of levulose from sucrose inversion. In an orange juice sample the sucrose was 72 and 92 per cent hydrolyzed in one- and two-months' storage periods, respectively, at 49°C., Curl (1947).

It would be expected that the reaction between ascorbic acid and sugars would take place with the carbonyl group of the latter and not with the alcohol groups. Hence, sucrose before hydrolysis would not be expected to react with ascorbic acid, since the carbonyl groups are both inactive. Sucrose would be expected to behave toward ascorbic acid in a manner similar to sorbitol, a hexahydric alcohol. When sorbitol was added (Pack 10) the losses were less than with sucrose, dextrose, or levulose and similar to those in packs without sugar (Packs 1 and 11). This is an indication that the inversion products of sucrose, and not sucrose itself, contribute to the loss of ascorbic acid.

The addition of 50, 150, and 500 mg. per 100 ml. of ascorbic acid (Packs 11, 12, and 13) in the absence of sugars resulted in an increasingly larger portion of the ascorbic acid being lost in plain tin cans. With 500 mg. per 100 ml. the percentage loss was twice that with 50 mg. per 100 ml. However, in the enamel cans the variations in concentration of the vitamin were without significant effect. It is noteworthy that in Pack 13 the losses of ascorbic acid were less in enamel than in plain tin cans, the only case where this was true. Of course, concentrations of 500 mg. per ml. are not encountered in citrus juices.

The percentage losses of ascorbic acid in a sample of orange juice stored at 49°C., Curl (1947), are also given (Table 2). Percentage losses in real orange juice in enamel were almost exactly the same as in Pack 7, which contained sucrose and 50 mg. per 100 ml. of ascorbic acid. Percentage losses in several other packs of synthetic mixtures were fairly close to those in the orange juice. Since the losses in orange juice were of the same order of magnitude as several of the synthetic packs, it is probable that the reactants involved are the same, or similar.

Color Density: Color density increases are given (Table 3) for Packs 1 to 6 only. In every case the color density increase (darkening) was greater in enamel cans than in the corresponding pack in plain tin cans. The combination of sugars and ascorbic acid (Pack 2) resulted in considerable darkening; the addition of amino acids to this mixture (Pack 4) resulted in even more darkening. The pack containing sugars and amino acids but no ascorbic acid (Pack 5) also darkened considerably — more than the one with sugars and ascorbic acid (Pack 2), but less than the one with amino acids, sugars, and ascorbic acid (Pack 4). Little darkening occurred in the absence of sugars and amino acids (Pack 1), of sugars (Pack 3), or of amino acids and ascorbic acid (Pack 6). The combination of sugars and amino acids was a principal cause of darkening, and the addition of ascorbic acid to this combination increased the effect.

Color density values for a sample of orange juice are also given (Table 3). Darkening in the orange juice was greater than in any of the synthetic mixtures tested, but was of the same order of magnitude as in the synthetic mixtures containing both sugars and amino acids. Values for Packs 7 to 13 are omitted because none of these contained amino acids, which together with sugars were chiefly responsible for the development of color.

SUMMARY

Ascorbic acid losses and darkening were measured in 13 packs of synthetic mixtures, analogous to orange juice, containing various combinations of ascorbic acid, sugars, and amino acids, and buffered to a pH of about 3.7 with citric acid and potassium citrate. The packs in enamel and plain tin cans were examined initially and after storage at 49°C. for 30 and 60 days.

Considerable losses of ascorbic acid occurred in the presence of buffer only. In this case disproportionation may have occurred. The addition of amino acids or sorbitol had little or no effect. In the mixtures containing sucrose the rate of loss of ascorbic acid was somewhat greater and was similar to that in orange juice. Dextrose had less effect than sucrose, and levulose had more effect than sucrose.

Losses of ascorbic acid were generally greater in enamel than in plain tin cans.

An increase in color density (darkening) was observed principally when both amino acids and sugars were present; the effect was more pronounced when ascorbic acid was also added. Color density increased only slightly in 60 days at 49°C. in buffered solutions containing only (1) ascorbic acid, (2) sugars, or (3) ascorbic acid plus amino acids. The color

density increase in the ascorbic acid-amino acid-sugar mixture was of the same order of magnitude, though somewhat less than in a sample of orange juice.

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THIAMIN, RIBOFLAVIN, AND NIACIN CONTENT OF RAW AND COOKED PORK FROM GRAIN-FED AND GARBAGE-FED PIGS[1]

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The present report is a continuation of the studies of Miller and Work (1946) who determined, by the rat growth method, the thiamin content of tissues from six pigs, three of which had been fattened on a diet containing large amounts of garbage. Their results consistently showed lower thiamin values for the tissues of the garbage-fed animals than for those of the grain-fed animals. This finding is in line with that of other workers, Pyke (1940), Hughes (1941), Miller, Pence, Dutcher, Ziegler, and McCarty (1943), Ellis and Madsen (1944), Pence, Miller, Dutcher and Ziegler (1945), and Heinemann, Ensminger, Cunha, and McCulloch (1946), all of whom have shown that the thiamin content of pork tissues is directly related to the thiamin content of the ration fed.

A number of workers, Ittner and Hughes (1941), Miller *et al.* (1943), Ellis and Madsen (1944), Pence *et al.* (1945), and Heinemann *et al.* (1946), have carried out similar studies with respect to riboflavin; and Christensen, Knowles, and Severson (1943) have published a preliminary report on niacin. Results of these studies indicate the pig has a much more limited capacity for storing riboflavin and niacin in the tissues than for storing thiamin.

In the work reported here thiamin, riboflavin, and niacin were determined by chemical methods on raw and cooked samples of the shoulder, loin, and liver of five grain-fed pigs and five garbage-fed pigs. Some samples were assayed before and after storage.

EXPERIMENTAL PROCEDURE

Experimental Pigs: Five pairs of pigs were used in this experiment. Each pair consisted of two animals from the same litter and (in all but one instance) of the same sex, which were placed on experiment at the same time and continued together on the experimental rations throughout the fattening period, which varied from 49 to 91 days, with one pair of very young animals having a fattening period of 191 days. One animal

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of each pair received a grain ration, while the other was fattened on garbage. The grain ration contained 83 parts rolled barley, eight parts meat meal, eight parts soybean oil meal, and one part salt. Thiamin content of these ingredients was determined in our laboratory. The garbage was from military camps and was of high quality. Similar garbage analyzed at this station had been shown to contain on the dry basis approximately 24 per cent crude protein, 29 per cent fat, and 39 per cent nitrogen-free matter, Maruyama, Henke, and Work (1944). Assays were made of the thiamin content of the garbage used in this experiment. Sixteen samples were assayed over a period of three months. In order to obtain representative samples, about a quart of wet garbage was removed from the lot being fed and run through a food grinder several times. The ground material was well mixed and aliquots were taken from the nearly homogeneous mixture for the determination of thiamin and moisture. Thiamin determinations were made on the wet sample and then calculated to the dry basis.

In addition to the basal ration, all animals received one pound of fresh panicum grass per day. The feeding experiment was conducted over the period from February, 1945, to January, 1946. Gains in weight varied from 96 pounds for the shortest fattening period to 166 pounds for the longest period. Garbage-fed pigs tended to gain more than grain-fed pigs. The weights of the 10 pigs at butchering varied from 173 to 263 pounds and averaged 238 pounds.

Sampling: The animals were slaughtered at the end of experimental periods and the two shoulders, the liver, and part of the loin were taken for sampling. Both cooked and raw samples were assayed. Shoulder cuts were prepared by the method recommended by the Committee to Standardize Methods of Cookery of the National Cooperative Experiment Station Project on "Conservation of the Nutritive Values of Foods" (1942). Standard methods for cooking loin chops or liver were not available. A description of the preparation of samples follows:

Shoulders—One cooked and one raw from each pig. The cooked shoulder was roasted whole at 177°C.(350°F.) to an internal temperature of 85°C.(185°F.). The lean meat of each shoulder was separated from the fat and bone, finely ground, mixed well, packed into small jars, and frozen.

Loin—Alternate pork chops were taken for the cooked and raw samples. The cooked samples were prepared by browning the chops in a hot pan for three minutes, then cooking for twenty-five minutes at low heat. The lean meat of both the cooked and raw samples was separated, ground, mixed, and frozen.

Liver—One-half of each liver was cut into slices one-quarter to one-half inch thick, which were browned for three minutes in a hot iron pan and cooked at low heat for 20 minutes more. Both the cooked and raw portions were ground, mixed, and frozen.

The samples were held in a freezing locker at —21 to —23°C.(—5 to —10°F.) until they were analyzed.

METHODS FOR VITAMIN ASSAYS

In the preparation of the samples for the chemical determination of the vitamins, a suspension was made by blending a weighed sample with water in a Waring Blendor. Aliquots of this suspension were then taken for digestion and subsequent analysis.

Thiamin: The thiochrome method was used. Samples were extracted by heating for one hour on the steam bath in 0.1 N sulfuric acid, then were digested with Clarase for three hours at 45°C.(113°F.) at pH 4.5. The filtered extracts of muscle tissues were assayed without further purification; liver extracts were further purified by adsorption on Decalso. The oxidation and extraction of thiochrome was carried out by an adaptation of the procedure of Mason and Williams (1942). A five-ml. aliquot of the extract or of the eluate from Decalso was pipetted into a large test tube (100 ml.). This was agitated by a stream of air through a bubbling tube and three ml. of alkaline ferricyanide solution added rapidly from a wide-tipped pipette. After 20 seconds of aeration, 20 ml. of isobutanol were added and the agitation continued for one minute. Later, the water layer was removed with suction and the isobutanol layer clarified by the addition of anhydrous sodium sulfate. Fluorescence of the extract was measured with a Coleman Model 12A Photofluorometer.

Riboflavin: The method of Peterson, Brady, and Shaw (1943) was used with a slight modification in the extraction procedure. Extracts were digested for three hours with pepsin in 9.5 N sulfuric acid, instead of with Clarase at pH 4.5 for 24 hours. The pH of the extracts was adjusted to between 4.5 and 5.5 before proceeding with the measurement of fluorescence. This procedure was adopted because experimental work in this laboratory showed that considerably higher values were obtained if the samples were digested with pepsin rather than with Clarase. Since much more complete digestion of the samples was obtained with pepsin, it seemed probable that a more thorough extraction was obtained by the use of this enzyme.

Niacin: This vitamin was determined by the method of Dann and Handler (1941) with some modification of the final color reaction, which was carried out as follows: To three ml. of the final clarified extract in a 50-ml. test tube was added one ml. of 10 per cent potassium dihydrogen phosphate and three ml. of water. The mixture was warmed in a water bath to from 75 to 80°C.(167 to 176°F.) after which two ml. of three per cent cyanogen bromide were added and the tube returned to the bath and held at from 75 to 80°C. for five minutes. During and after the addition of cyanogen bromide, the tube was carefully protected from daylight; however, artificial light was used in the laboratory. The color, which was measured with an Evelyn Colorimeter, was produced at the colorimeter. To the tube, which had been cooled to room temperature, were added two ml. of an acid solution of p-aminoacetophenone (10 g. plus 60 ml. concentrated hydrochloric acid diluted to 200 ml.). Contents of the tube were given a quick shake, poured into a colorimeter tube, and read in the colorimeter, using a 420 filter. Two blanks were read, a reagent and a sample blank. The reagent blank was used to set the galvanometer to 100 before reading the samples. The L value for the sample blank (read with the galvanometer set to 100 with water) was subtracted from the L value for the corresponding sample, thus correcting for any extraneous color in the extracts. This procedure is rapid and just as sensitive as the reaction with metol, as described by Dann and Handler.

RELIABILITY OF METHODS USED

In order to check on the reliability of the chemical methods, two procedures were used: (1) a number of the meat samples were assayed for thiamin and riboflavin by the rat-growth method, and (2) the chemical methods for the three vitamins were applied to two reference samples which had been assayed by six other laboratories in a collaborative study.

The rat-growth method used in this laboratory for the bio-assay of thiamin has been described elsewhere, Miller (1943).

For the riboflavin assays, the following basal diet was used: Casein 18 per cent, cornstarch or Cerelose 68 per cent, cottonseed oil five per cent, corn oil three per cent, codliver oil two per cent, and salt mixture four per cent. B vitamins were supplied by the addition of eight mg. thiamin hydrochloride, eight mg. pyridoxine, 100 mg. calcium pantothenate, 600 mg. choline, and 100 mg. nicotinamide per kilogram of diet. Animals were placed on the diet at a weight of 40 to 50 grams and depleted to constant weight. Controls were fed pure riboflavin solution at two levels, four and eight micrograms daily. Supplements of riboflavin or meat were fed three or four times a week for three weeks.

DETERMINATION OF COOKING LOSSES AND STORAGE LOSSES

Moisture and ether extract determinations were made on all the meat samples and the vitamin values were converted to the fat-free-dry basis for use in calculating vitamin losses resulting from cooking.

Vitamin determinations were repeated on a number of samples which had been in the freezing locker for from 10 to 12 months. This was done as a check on the stability of the vitamins during storage, since some of the samples had been held several months before assaying. Error due to any change which might occur during this period was, however, minimized for our purposes by always assaying the samples from both pigs of any one pair at the same time.

RESULTS AND DISCUSSION

Comparison of Methods of Assay: Results of our analysis of reference samples for thiamin and niacin agreed very well with those obtained by other laboratories, but our values for riboflavin were considerably higher.

Thiamin values of four pork samples determined by biological assay in this laboratory were in good agreement with those obtained by the chemical method. They ranged from 83 to 100 per cent of the chemical values, with an average of 91 per cent.

Biological assay of 11 meat samples for riboflavin, however, showed values higher than those obtained by the chemical method. Values of nine samples of muscle tissue ranged from 94 to 154 per cent of those obtained by the chemical method, with an average of 132 per cent. Biological assay of two samples of liver yielded results in excellent agreement—101 and 105 per cent of those determined by chemical methods.

Other workers have observed lack of agreement between results of chemical and biological assays. Emmett *et al.* (1941) compared biological, microbiological, and fluorometric methods of determining riboflavin content of concentrated extracts. Their fluorometric determinations varied

from 60 to 121 per cent of the values obtained by the rat growth method, with an average of 105.3 per cent. Variability was greatest on samples of low potency. Sullivan *et al.* (1943) determined the riboflavin content of buttermilk by several methods. These workers found agreement among the methods to be variable and a tendency for the rat-growth method to yield higher values than the fluorometric. More work needs to be done on techniques of the rat-growth method in order to explain these discrepancies.

Our studies show that the chemical methods used by us for thiamin and niacin give values in good agreement with those obtained in other laboratories. The method of thiamin assay compares well with the rat-growth method used here. The method used for riboflavin, however, gives values higher than those obtained by other workers with chemical methods, but lower than those obtained in our laboratory by the rat-growth method.

Effect of Ration: Vitamin values for the three cuts of meat are shown (Table 1). No differences were found between the two groups of pigs for any of the vitamins except thiamin. Here the differences were large—tissues from the garbage-fed animals contained only 41, 41, and 68 per cent as much thiamin in shoulder, loin, and liver, respectively, as those from grain-fed animals. This difference is in line with the difference in rations. The grain ration was estimated to contain from three to four micrograms thiamin per gram, or 1,300 to 1,800 micrograms per pound. The average value found for the thiamin content of the garbage was 2.1 ± 0.16 micrograms per gram on the dry basis, or 945 micrograms per pound.

TABLE 1

Thiamin, Riboflavin, and Niacin Contents of Raw Pork Tissues From Grain-Fed and Garbage-Fed Hogs (Fresh-Weight Basis)

Tissue	Thiamin		Riboflavin		Niacin	
	Grain-fed	Garbage-fed	Grain-fed	Garbage-fed	Grain-fed	Garbage-fed
	μg./gm.	μg./gm.	μg./gm.	μg./gm.	μg./gm.	μg./gm.
Shoulder	7.8±0.7[1]	3.3±0.2	2.5±0.1	2.6±0.1	53.7± 2.5	53.0±1.9
Loin	7.5±0.3	3.1±0.1	2.0±0.1	2.1±0.1	58.7± 2.7	56.4±2.5
Liver	2.5±0.2	1.7±0.1	28.1±2.0	29.1±1.2	154 ±10.0	173 ±5.0

[1] Standard error.

Thiamin values for the samples from garbage-fed pigs agree with previous values obtained at this station by Miller and Work (1946), who reported cooked shoulder tissues from garbage-fed pigs to contain 3.4 micrograms per gram. Their values for grain-fed pork are lower than the present ones, however. The grain ration used by them was not analyzed for thiamin content; it contained molasses and tankage and less barley than the ration used for this study.

Thiamin values for the samples from grain-fed pigs agree well with those of Miller *et al.* (1943), at Pennsylvania State College, who found 7.9, 9.5, and 3.3 micrograms per gram for shoulder, loin, and liver, respectively, from pigs fattened on a grain ration providing 1,315 micrograms of thiamin per pound.

Values for riboflavin and niacin were the same in tissues from grain-fed and from garbage-fed pigs. This is in line with the findings of other

workers that the pig has a limited capacity for storing riboflavin and niacin. Since the values are in good agreement with the values for pork found in the literature, it seems probable that both the garbage and the grain ration supplied adequate amounts of these two vitamins.

Effect of Cooking: Percentages of moisture and ether extract found in the cooked and raw samples are shown (Table 2). Because the cooked samples contained less water and more fat than the raw samples, all vitamin values were calculated to the fat-free-dry basis for estimation of cooking losses. No significant differences were found for either of these constituents between the two groups of pigs. Ether extract values did run somewhat higher for the garbage-fed animals. This difference might have been expected, since the garbage had a high fat content, about 30 per cent on the dry basis.

TABLE 2

Moisture and Ether Extract in Raw and Cooked Pork Tissues

Tissue	Moisture		Ether extract	
	Grain-fed	Garbage-fed	Grain-fed	Garbage-fed
	pct.	*pct.*	*pct.*	*pct.*
Raw				
Shoulder	73.3±0.4[1]	72.9±0.9	5.3±0.4	5.9±0.9
Loin	69.3±0.2	68.8±1.5	7.5±0.3	8.7±0.3
Liver	69.9±0.7	69.3±0.7	2.2±0.2	3.1±0.3
Cooked				
Shoulder	54.7±1.3	55.5±0.7	12.4±1.5	11.7±0.7
Loin	47.0±2.5	47.0±1.2	16.9±3.0	17.9±2.9
Liver	56.1±1.3	58.2±1.2	5.7±0.2	6.8±0.5

[1] Standard error.

Cooking losses resulting from the methods used are shown (Table 3). Losses of thiamin were large for all samples—60 per cent in roast shoulder, 40 per cent in fried pork chops, and about 20 per cent in fried liver. The large losses in shoulder are undoubtedly due to the long cooking time (three and one-half to five hours for shoulders weighing from eight to 14 pounds). In general, the greatest losses occurred in the largest shoulders, which required longer periods of cooking.

Losses of niacin were not so severe as the thiamin losses, but were appreciable, averaging 34 per cent for roast shoulder, 25 per cent for fried pork chops, and 11 per cent for fried liver. These losses, as well as those for thiamin, are somewhat greater than those generally obtained by other workers.

Riboflavin showed no consistent loss as a result of cooking; the calculated retention varied from 87 to 123 per cent and averaged 100 per cent. This is in contrast to the findings of McIntire, Schweigert, Henderson, and Elvehjem (1943), Brady, Peterson, and Shaw (1944), Cover, McClaren, and Pearson (1944), and Sarett and Cheldelin (1945), who have reported cooking losses of riboflavin in meat of from 15 to 30 per cent. Others, however, Rice and Robinson (1944), Richardson, Mayfield, and Hedrick (1945), Noble, Gordon, and Nelson (1946), and Noble and Gordon (1947), have found apparent increases (some quite large) in riboflavin as

a result of cooking or canning. Usually these increases have been largest in cases where the meat was braised or canned, that is, in processes where the meat was well-cooked.

TABLE 3

Retention of Thiamin, Riboflavin, and Niacin in Cooked Pork, Fat-Free Dry Basis

Tissue	Thiamin		
	Raw	Cooked	Retention
	μg./gm.	*μg./gm.*	*pct.*
Shoulder			
Grain-fed	36.2± 3.3[1]	14.3± 1.3	40±2.4
Garbage-fed	15.3± 1.9	6.3± 1.3	41±1.7
Loin			
Grain-fed	32.3± 3.5	19.0± 0.4	59±4.1
Garbage-fed	13.8± 0.7	8.2± 0.7	59±3.1
Liver			
Grain-fed	9.2± 0.8	7.0± 0.7	76±6.2
Garbage-fed	6.3± 0.5	5.6± 0.6	89±4.3

Tissue	Riboflavin		
	Raw	Cooked	Retention
	μg./gm.	*μg./gm.*	*pct.*
Shoulder			
Grain-fed	11.5± 0.8	11.5± 0.4	100±3.8
Garbage-fed	12.3± 0.2	11.8± 0.6	96±4.0
Loin			
Grain-fed	8.3± 0.7	8.4± 0.7	101±3.5
Garbage-fed	9.1± 0.5	9.0± 0.6	99±3.0
Liver			
Grain-fed	102 ± 6.7	104 ± 6.9	102±4.3
Garbage-fed	106 ± 4.4	108 ± 4.5	102±5.6

Tissue	Niacin		
	Raw	Cooked	Retention
	μg./gm.	*μg./gm.*	*pct.*
Shoulder			
Grain-fed	251 ±10	162 ± 8	65±2.2
Garbage-fed	250 ± 8	167 ± 6	67±1.3
Loin			
Grain-fed	254 ± 9	191 ± 5	75±3.6
Garbage-fed	253 ±10	191 ± 8	75±0.9
Liver			
Grain-fed	560 ±35	492 ±32	88±2.6
Garbage-fed	628 ±20	567 ±20	90±1.7

[1] Standard error.

Hinman *et al.* (1946) made a careful study of this phenomenon. They used both fluorometric and microbiological methods and carefully checked the methods of calculating retention. No sources of error were found which could account for the high recoveries, and the suggestion was made that certain cooking procedures may liberate riboflavin from a precursor or complex from which it is not readily liberated by the methods of extraction usually employed.

Results obtained in our laboratory seem reasonable in the light of this idea. In our preliminary work, using Clarase to digest the samples, retentions of over 100 per cent of riboflavin in cooked samples were obtained. When pepsin was used, the values in micrograms per gram were consid-

erably higher for both cooked and raw samples and the calculated retention of riboflavin in cooked samples averaged 100 per cent. Apparently digestion with pepsin in acid solution (0.5 N H_2SO_4) liberates bound riboflavin much more effectively than does digestion with Clarase at pH 4.5.

Effect of Storage: Sixteen samples (12 muscle and four liver; eight raw and eight cooked) were reassayed for the three vitamins after storage in the freezing locker for five to nine months beyond the original assay. Little or no loss occurred during this time as shown by the data (Table 4). Agreement between the two series is excellent for both riboflavin and niacin and it may be concluded that no loss of these vitamins occurred during storage. Results indicate a slight loss of thiamin in shoulder and loin tissues, but no loss in liver.

TABLE 4

Retention of Vitamins in Pork Samples During Storage in Freezing Locker at —13°C.(8°F.)

Tissue	Storage time	No. of samples	Vitamin retention		
			Thiamin	Riboflavin	Niacin
	mo.		*pct.*	*pct.*	*pct.*
Shoulder and chops	5 to 6	6	85(79 - 93)	94(84-100)	98(94-101)
Shoulder and chops	6 to 9	6	92(88 -100)	100(86-119)	98(97-100)
Liver	5 to 6	2	106(105-106)	100(96-104)	98(97- 99)
Liver	6 to 9	2	95(93- 96)	96(90-101)	98(96- 99)

SUMMARY

Tissues of five pairs of pigs, in which one of each pair received a grain ration and the other garbage, were assayed by chemical methods for thiamin, riboflavin, and niacin.

Values for thiamin were much lower in the garbage-fed pigs than in the grain-fed animals, averaging 3.3, 3.1, and 1.7 micrograms per gram for shoulder, loin, and liver, respectively, as compared with 8.0, 7.5, and 2.5 micrograms per gram for the grain-fed animals.

There was no difference between the two groups with respect to riboflavin and niacin. Average values found were 2.3 and 23.6 micrograms of riboflavin per gram for muscle and liver, respectively, and 56 and 165 micrograms per gram for niacin in muscle and liver.

Cooking and storage losses were also determined. Losses of thiamin were greatest—60 per cent in roasting the whole shoulder and 40 per cent in frying pork chops. Niacin also showed considerable loss; 35 per cent in roasting the whole shoulder and 25 per cent in frying pork chops. No consistent cooking losses of riboflavin were found. Storage of the ground samples in a freezing locker for from five to nine months at —5 to —10°F. resulted in no loss of either riboflavin or niacin and little or no loss of thiamin.

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EFFECT OF STORAGE ON VITAMINS AND QUALITY IN CANNED FOODS[1]

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In modern civilization, canned foods are important from both the dietary and economic viewpoints. The importance of this class of foods is dependent in no small measure on its comparatively high stability. Although heat processed foods are relatively stable, it is, nevertheless, generally recognized that in many instances quality and vitamin content may not be completely independent of storage conditions.

As early as 1924, the possible influence of holding on the vitamin C content was recognized, as evidenced in a study by Kohman *et al.* (1924) showing that the antiascorbietic value of canned apples remained essentially unchanged during storage for eight months. Other early studies employing bio-assay techniques suggested that the vitamin C activities of canned citrus and tomato products also are stable during storage periods of several months, Clow and Marlott (1930), Delf (1924), Fellers and Isham (1932). As rapid chemical or microbiological assay methods for determining vitamin values of foods became available, an active interest in the more exact establishment of the influence of storage on vitamins in canned foods gradually developed. As a result, the stabilities of the better-known vitamins in canned foods during storage and the major factors influencing their retentions are currently under active investigation.

ASCORBIC ACID

Ascorbic acid is easily oxidized to dehydroascorbic acid by atmospheric oxygen in the presence of suitable catalysts. In the storage of raw foods, in the cooking and holding of cooked foods, and in canning and the early stages of storage of canned foods, the degree of ascorbic acid destruction appears to be associated with oxidation. The influence of storing canned foods on ascorbic acid will be considered in two periods, the first few days after canning and subsequent prolonged holding.

Short Storage: Depending upon the product under consideration, an appreciable quantity of oxygen may be occluded in the product or trapped in the headspace of the container in which foods are sealed. It is believed that reaction of the tin and steel of the container with the product uses up the free oxygen, producing a highly reduced system, within a few days after packing in plain tin cans. In the case of non-metallic containers the product combines with the oxygen to produce anaerobic conditions. Theoretically, therefore, consumption of free oxygen by the container should be favorable to ascorbic acid retention.

In tests with home-canned tomatoes and tomato juice, Daniel and Rutherford (1936) observed better ascorbic acid retention during canning

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and storage for samples in tin cans as compared to glass jars. Observations by other investigators, Hauck (1938), Lueck and Pilcher (1941), McConnell *et al.* (1945), have confirmed better retention of ascorbic acid in products in plain tin cans as compared to glass. Tressler and Curran (1938) present data which they interpret as demonstrating that the headspace oxygen is the important factor influencing the difference in plain tin and glass containers with respect to ascorbic acid retention. The fill of the container and venting out of air is considered by Esselen and Woodward (1945) to be a factor influencing the difference in ascorbic acid values for tomato juice in tin and glass. Headspace oxygen has also been observed to affect ascorbic acid retention in bottled berry juices, Beattie *et al.* (1943). However, under practical conditions, it is difficult to avoid sealing small amounts of atmospheric oxygen in the container.

During the period that any free oxygen sealed in the container is being consumed, as during the first month of storage after packing, ascorbic acid destruction under some conditions appears to be more rapid than in subsequent storage, Clayton *et al.* (1944), Daniel and Rutherford (1936), Hauck (1938), Riester *et al.* (1945).

This laboratory and other groups of investigators have advanced the thesis that the reducing action of the tinplate competes with the product for the small amount of free oxygen in the sealed container. Since glass does not possess similar reducing properties, the product must use up any oxygen sealed in the package. Data presented by Lueck and Pilcher (1941) demonstrate the protective action of tinplate toward ascorbic acid in grapefruit juice and tomato juice. Studies by Hauck (1938, 1943), Reynolds (1938), Clayton *et al.* (1944), Moore *et al.* (1944), all present data which support this thesis. The superior retention of ascorbic acid in citrus and tomato products appears to be associated with contact with the tinplate rather than to differences in headspace, exposure to light, or interference by tin or iron salts in the analysis.

Coating the interior of the can with enamel minimized the surface exposed to the product and the amount of tin taken up by the product in early storage. Adam (1941) observed vitamin C declines of 20 to 30 per cent due to the oxygen in the headspace of some English canned foods and states that plain cans are to be preferred to lacquered cans, where they can be used, because of the protection to vitamin C.

Tests conducted in this laboratory during the past several years indicate that under certain experimental conditions which are seldom attained in actual commercial practice, plain cans afford greater protection to vitamin C than enamel-lined cans for some products. Ten months after canning, tomato juice in plain cans was found to contain 15.6 mg. of ascorbic acid per 100 ml. as compared to 13.0 mg. from juice from the same 1,000-gallon tank, but packed in enamel-lined cans. The differences in values were observed during the first 30-day period. The higher ascorbic acid values found by titration were in agreement with bio-assay data. In the case of tomato juice produced by methods which incorporate considerable quantities of air in the sealed enamel-lined cans, decreases of as much as five mg. of ascorbic acid per 100 ml. may occur in the first three weeks after canning. Under similar conditions, juice packed in plain cans exhib-

ited much better ascorbic acid retention. However, tomato juice prepared and packed in enamel-lined cans under conditions which held at a minimum air sealed in the can, exhibited ascorbic acid decreases of less than one mg. per 100 ml. during similar holding. Under conditions which avoid sealing appreciable quantities of oxygen in the can, enamel-lined and plain tin cans are essentially equivalent for the retention of ascorbic acid in citrus juices, Riester *et al.* (1945). In another laboratory, storage of grapefruit juice, from different canneries, for 40 days at 22°C.(72°F.) resulted in an average ascorbic acid loss of nine per cent, Wagner *et al.* (1945).

Enamel-lined and plain cans were found (Pilcher and Feaster) to be essentially equivalent with respect to conservation of ascorbic acid in peas during storage, suggesting that type of container may not be a factor influencing ascorbic acid retention in the less acid vegetables.

Prolonged Storage: Although some decline of ascorbic acid may be associated with long storage of canned foods, Guerrant *et al.* (1945), Lamb (1946), Olliver (1936), and Riester *et al.* 1945), the extent of the decline after the first few weeks appears to be associated with the type of the product and the storage temperature.

Roberts (1937) reported a decrease of 25 per cent in canned grapefruit juice stored at room temperature for 15 months. Holding citrus juice for one year at 9, 24, or 37°C.(48, 75, or 98°F.) was found by Ross (1944) to result in ascorbic acid retentions of 94, 75, and 17 per cent, respectively.

At 27°C.(80°F.) for one year, which is probably more severe than commercial storage conditions for canned foods, citrus juices retained 70 to 80 per cent of their ascorbic acid content, Moschette *et al.* (1947). At 10 and 18°C.(50 and 65°F.) ascorbic acid retention was decidedly better. According to Lamb (1946), storage at 21°C.(70°F.) results in approximately a one per cent per month decrease in the ascorbic acid in citrus juices.

At an average temperature of approximately 38°C.(100°F.), which is well above the sustained temperature at which canned foods are usually stored for domestic consumption, as much as 200 mg. of ascorbic acid may disappear from a No. 2 (20-ounce) can of orange juice in one year. It has been reported, Moore *et al.* (1944), that in stored juice, the dehydroascorbic acid content does not exceed two per cent of the total. The mechanism of the reactions involved ir this type of destruction has not, as yet, been established. In view of observations by Beattie *et al.* (1943) that the increase in yellow color in berry juice during storage may progress at somewhat the same rate as loss of ascorbic acid, it would be of interest and certainly of importance to know the end-products of ascorbic acid destruction.

At a temperature of 43°C. (110°F.), which might simulate tropical storage in wartime, but which is far above domestic storage conditions, tomato juice declined to 25 per cent of its ascorbic acid in a 12-month period, Guerrant *et al.* (1945). In the region of 27 to 29.5°C.(80 to 85°F.), the upper range of temperatures to which canned foods are likely to be exposed for long periods in this country, 75 to 85 per cent ascorbic acid retentions were observed at the end of one year, Guerrant *et al.* (1945),

Moschette *et al.* (1947). Another series of tests, Sale (1946), indicates that storage for one year at room temperature 19 to 32°C.(66 to 90°F.) results in average ascorbic acid decreases of 4.3 mg. per 100 ml., or 21 per cent of the original. At refrigerated temperatures of —1 to 10°C. (30 to 50°F.) retentions of 90 per cent or above were found at the end of 12 months, Guerrant *et al.* (1945), Moschette *et al.* (1947), Sale (1946).

In practice, tomato juice and other canned foods are not held under conditions of constant temperatures. At the time of packing, the cans are cooled to 32 to 43°C.(90 to 110°F.) to provide sufficient residual heat to dry the metal cans. Many products are immediately cased and stacked in warehouses at these temperatures. During the past year, the influence of this procedure on vitamin content and quality of tomato juice has been investigated.

TABLE 1

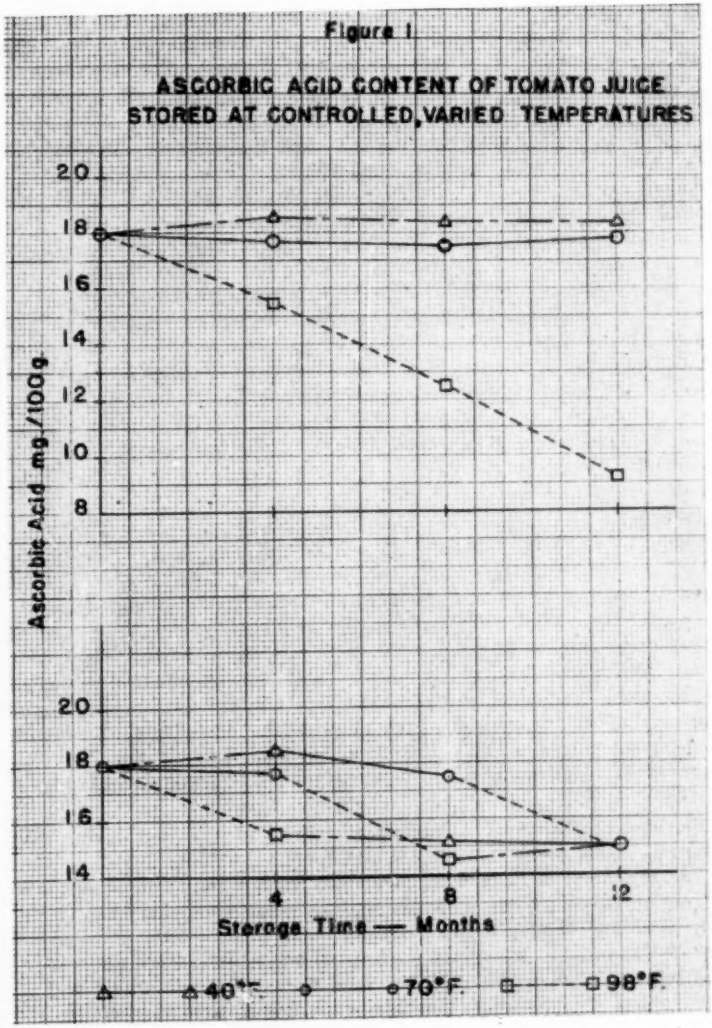
Comparison of Warehouse and Controlled Storage on Ascorbic Acid and Thiamin Retention in Tomato Juice

Storage conditions		Ascorbic acid		Thiamin	
Location	Months	Mg./ 100 gm.	Retention pct.	Mg./ 100 gm.	Retention pct.
46-ounce cans cased at 110 to 115°F.					
Initial Examination	0.1	15.4	....	0.041	....
Center of stack	4	13.4	87	0.039	95
Top of stack	4	13.4	87	0.041	100
Side of stack	4	14.0	91	0.038	93
On floor beside stack	4	15.3	99	0.045	109
40°F. refrigerator	4	15.1	98	0.046	112
70°F. room	4	14.8	96	0.042	102
No. 2 cans cased at 103 to 108°F.					
Initial Examination	0.1	15.3	....	0.046	....
Center of stack	4	13.0	85	0.041	89
Top of stack	4	13.6	89	0.038	83
Side of stack	4	13.5	88	0.039	85
On floor beside stack	4	14.1	92	0.042	91
40°F. refrigerator	4	14.5	95	0.041	89
70°F. room	4	13.7	90	0.042	91

In an experiment carried out during the 1946 season, consecutive cases of 46-ounce (404 by 700) cans of tomato juice were removed from a commercial production line. One case was further cooled and samples taken for initial analysis and storage at controlled temperatures of 4 and 21°C.(40 and 70°F.). Other cases were placed in the stack (19 cases long by 7 wide by 22 high) of juice in the warehouse. One case was placed near the center, one near the top, one near the outside of the stack, and one on the floor beside the stack so that air could freely circulate around it. At the time of casing and stacking, the temperature of the product was 43 to 46°C.(110 to 115°F.). A similar stack was prepared from No. 2 (307 by 409) cans cased and stacked at approximately 41°C.(105°F.).

The samples remained in the stacks for about four months after which they were removed from the warehouse for examination. Results of these examinations are summarized (Table 1). During the period that any

Figure 1


ASCORBIC ACID CONTENT OF TOMATO JUICE
STORED AT CONTROLLED, VARIED TEMPERATURES
Ascorbic Acid mg./100g.
20
18
16
14
12
10
8
20
18
16
14
4
8
12
Storage Time — Months
40°F.
70°F.
98°F.

oxygen entrapped in the sealed cans was being consumed and the large block of canned juice was approaching the temperature of the surrounding air, a maximum decrease in ascorbic acid content of 15 per cent was observed for a sample from the center of the stack. Retentions in samples stored at 4 and 21°C.(40 and 70°F.) or on the floor beside the stacks were about 10 per cent better. These data indicate that stacking of warm tomato juice in large blocks may exert a somewhat unfavorable effect on ascorbic acid retention.

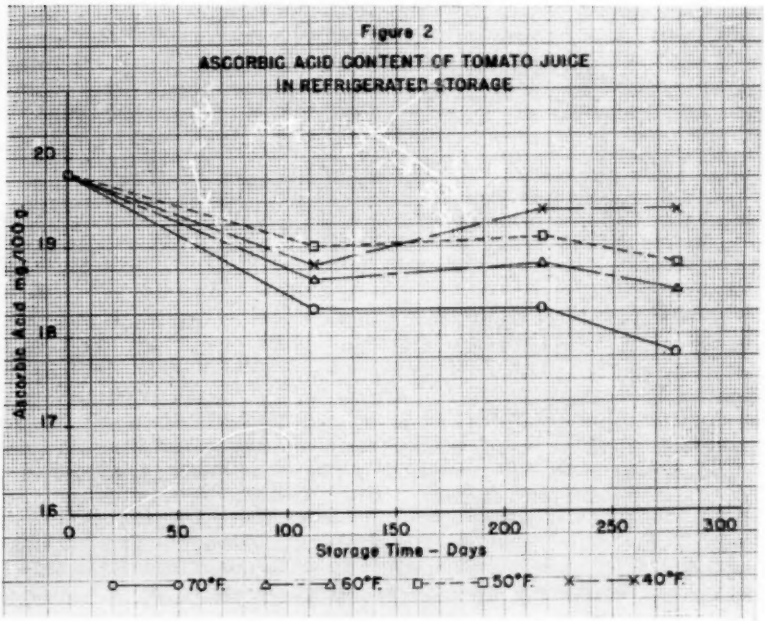
To obtain further information regarding the effect of varied temperatures on ascorbic acid retention, tomato juice from one uniform lot was placed in storage at 4, 21, and 37°C.(40, 70, and 98°F.). At four-month intervals, the temperatures of storage of certain samples were shifted. The schedule followed provided samples held at controlled temperatures of 4, 21, and 37°C.(40, 70, and 98°F.) for one year and samples stored four months at each of these temperatures. Results of these tests, summarized (Fig. 1) show excellent retention at constant temperature storage of 4 or 21°C.(40 or 70°F.), but a retention of only 50 per cent at 37°C. (98°F.). In case of samples held four months at each of the three temperatures, the influence of four months at 37°C.(98°F.) was predominant with respect to ascorbic acid retention. At this higher temperature, a decrease of approximately three mg. was observed in a four-month period. The sequence of storage at the various temperatures did not influence materially the rate of decline.

It is well established that small sacrifices of ascorbic acid may accompany 21°C.(70°F.) storage of several canned foods. Therefore, the influence of 4, 10, 16, and 21°C.(40, 50, 60, and 70°F.) storage on vitamins and quality of tomato juice was investigated. Changes in ascorbic acid contents through 279 days are illustrated (Fig. 2). At any of these temperatures, retention was 90 per cent or better during nine months' storage. Juice stored at 4°C. contained only 1.6 mg. per 100 grams more ascorbic acid than juice stored at 21°C. Similar tests now in progress indicate that at the end of three months, the ascorbic acid content of orange juice stored at 21°C. has decreased by 1.4 mg. per 100 ml., as compared to a decline of 0.5 mg. per 100 ml. for companion samples held at —18°C.(0°F.).

Published information regarding ascorbic acid retention during storage of canned foods other than citrus and tomato products is rather limited. During the first 30 days, high temperature was found to accelerate the destruction of this vitamin in green lima beans and whole kernel yellow corn, Guerrant *et al.* (1945). Beyond this point, —1, 6, 29, and 43°C. (30, 42, 85, and 110°F.), temperatures were found to have essentially the same influences on retention. However, storage beyond nine months was accompanied by apparent increases in ascorbic acid, especially at the higher temperatures, Guerrant *et al.* (1945). It was suggested that these apparently anomalous values appeared to be due to the formation of non-ascorbic acid compounds which reacted with the dye during the course of the assay.

In peaches, sliced pineapple, pineapple juice, tomatoes, and grapefruit segments, ascorbic acid retentions were 70 per cent or better, Moschette

et al. (1947), during one year at 27°C.(80°F.). At 18°C.(65°F.), which is probably near the year-round average storage temperatures in most sections of the country, retentions were generally 85 to 95 per cent after one year. Storage at 10°C.(50°F.) was accompanied by slightly better retentions.



A decrease of 2.8 mg. per 100 grams of ascorbic acid in canned peas between the sixth and ninth month of storage at room temperature, Mahoney *et al.* (1946), appears atypical and may not be indicative of the rate of decline in this product. Tests conducted in this laboratory some ten years ago indicate that ascorbic acid in canned peas is quite stable, 85 per cent or more being retained during one year at temperatures up to 37°C.(98°F.). Additional reports, Adam (1941), Clayton *et al.* (1944), Josephson and Doan (1945), Olliver (1936), Pederson and Beattie (1946), indicate that some decline of ascorbic acid accompanies storage of a variety of canned foods. Rate of decline is probably influenced by storage temperature.

CAROTENE AND VITAMIN A

Using a bio-assay technique, Swanson *et al.* (1940) investigated the influence of storage at room temperature on the vitamin A activity of home-canned tomatoes. Over a period of 42 months, the change in vitamin A activity was not appreciable. DeFelice and Fellers (1938), using a colorimetric assay procedure, obtained information indicating that holding

for three months did not adversely influence the carotene content of canned spinach.

The influence of canning and subsequent storage on carotene in asparagus, green beans, corn, and peas was the subject of an investigation by McConnell and co-workers (1945). According to their observations, carotene was quite stable in these canned vegetables. Neither the type of container nor exposure to light in normal handling had a significant effect on carotene stability.

A recent report, Guerrant *et al.* (1945), indicates a rather gradual and continuous decrease in carotene of canned green lima beans and yellow corn at temperatures as low as —1°C.(30°F.). At elevated temperatures, 29 and 43°C.(85 and 110°F.), the decline was somewhat greater. However, the influence of high temperature storage on carotene was not nearly as marked as that on ascorbic acid and thiamin. Storage of tomato juice for six months at low temperatures affects the carotene only slightly. However, in the second six months' period, definite decreases of carotene were observed for samples at —1, 6, 29, and 43°C.(30, 42, 85, and 110°F.), Guerrant *et al.* (1945).

In contrast to the above data, Moschette *et al.* (1947) observed that canned tomato juice at the end of 12 months (10, 18, or 27°C.) contained essentially the same amount of carotene as when first placed in storage. Carotene in peaches, tomatoes, and tomato juice was retained to the extent of 86 to 100 per cent after one year at these three temperatures. As the temperature was increased, a slight tendency toward lowered carotene retentions was observed. Analytical methods used did not differentiate the different isomers of carotene.

VITAMIN D

The vitamin D activity of canned foods other than fortified evaporated milk and certain of the marine products is low. Hence, the influence of storage on this vitamin in fruit and vegetable products has not been studied. However, Krieger and Scott (1938) followed the vitamin D activity of irradiated evaporated milk for a period of three years. During the first year, which is considered the normal life of this product, no loss of vitamin D potency was observed. Even at the end of three years under moderate temperature, little or no loss of activity had occurred.

THIAMIN

Thiamin may be readily destroyed under numerous conditions, hence, it is of interest to consider the fate of this vitamin during storage of typical canned foods. Using bio-assay techniques, Schlutz and Knott (1939) and Knott (1942) demonstrated that holding may result in definite decreases in thiamin contents of evaporated milk. Likewise, the thiamin content of heat-processed pork luncheon meat has been shown, Feaster *et al.* (1946), Rice and Robinson (1944), to be influenced by storage conditions. At exaggerated temperatures, approximately 38°C.(100°F.), declines of the order of 80 to 90 per cent were observed in one year. At more reasonable temperatures, with respect to domestic storage (21 to 27°C.) retentions of 55 to 75 per cent were found for one year's holding.

Refrigeration permitted thiamin survivals in this product of 90 to 100 per cent at the end of 12 months.

Changes in thiamin values of typical canned fruit and vegetable products during storage have been studied, Guerrant *et al.* (1945), Moschette *et al.* (1947). Abnormally high temperature (43°C.) was observed to permit thiamin survivals of 20 to 25 per cent in green lima beans and yellow corn, Guerrant *et al.* (1945). At 29°C.(85°F.), a definite decrease was observed during one year. Low temperature holding (—1 and 6°C.) permitted the thiamin values of these products to remain essentially unchanged during one year. Another study, Clifcorn and Heberlein (1944), indicates that storage of canned vegetables for a short time may result in decreases in thiamin values.

In an investigation of the influence of storage on thiamin survival of the more acid types of canned foods (citrus juices, pineapple, peaches, and tomato products) 27°C.(80°F.) storage was observed to result in decreases of the order of 10 to 20 per cent in one year, Moschette *et al.* (1947). At 10 or 18°C.(50 or 65°F.), thiamin survivals were quite similar and above 90 per cent.

Guerrant *et al.* (1945) observed that temperature is a major factor influencing thiamin survival in tomato juice during storage. At —1 and 6°C. thiamin values remained essentially unchanged during one year. A definite decline was observed during one year at 29°C. with thiamin survival of 31 per cent after one year at 43°C.(110°F.).

Tests conducted in this laboratory indicate that differences in thiamin survival in tomato juice during six months' storage at 4 and 21°C. (40 and 70°F.) are extremely small. Data (Table 1) indicate thiamin declines of zero to 17 per cent during four months in commercial warehouse stacks. However, in this particular series of samples, a decrease of 11 per cent in thiamin values was noted during storage for four months at 4°C. On this basis, the decrease in thiamin value of the warehouse sample was only six per cent greater than that of a sample at 4°C. This suggests that during the period that stacks of tomato juice in a commercial warehouse are cooling to room temperature, thiamin content is not seriously affected.

B VITAMINS OTHER THAN THIAMIN

Within the past few years, a limited number of publications have appeared presenting information on the influence of storage on niacin, riboflavin, and pantothenic acid in typical canned foods. Decreases in riboflavin contents of tomato juice, green lima beans, and whole kernel yellow corn were observed during storage for one year. At the extreme temperature of 43°C., 60 per cent of the riboflavin present in tomato juice at the beginning of storage remained after one year, Guerrant *et al.* (1945). At a temperature of 29°C. riboflavin retentions of the order of 75 to 90 per cent were observed, while at —1 or 6°C. retentions approached 100 per cent at the end of one year.

The riboflavin in tomatoes and tomato juice appears to be rather stable during storage of the canned product. At the end of one year, these products stored at 10, 18, and 27°C.(50, 65, and 80°F.) retained 83 to 95 per cent of the riboflavin present when placed in storage, Moschette *et al.*

(1947). In this range, temperature had a very slight influence on this vitamin.

Storage of canned pork products for periods of six months to a year, Feaster *et al.* (1946), Rice and Robinson (1944), had little adverse effect on the riboflavin content of this product. In the range of 7 to 37°C. (45 to 98°F.) temperature of storage did not appear to be an important factor. However, in one study, Feaster *et al.* (1946), riboflavin increases of as much as 35 per cent were observed during the first three months after packing. A microbiological assay procedure was used in these tests.

Recently, Ives of this laboratory has experienced difficulty in attempting to follow the riboflavin content of canned vegetables during storage, using a microbiological assay. At successive examination dates, the riboflavin values of yellow corn, spinach, lima beans, and other canned vegetable products showed sizeable apparent increases. In fact, at the end of 18 months' storage, the riboflavin contents of certain products were found to be two and one-half times those observed at the initial examination. A careful check of standards, the strain of organisms used, and other points of technique failed to reveal the reason for the observed increases. In tests with certain dairy products in progress at the same time, no increases in riboflavin were observed to accompany storage. These observations suggest that extraction of riboflavin from the samples studied was not complete or uniform at the different assays. The possibility of growth-inhibiting factors in these samples, which disappeared during storage, or the formation of growth-stimulating substances during storage, also cannot be ruled out. Further study of these observed apparent increases in riboflavin contents of canned vegetables during storage is strongly suggested and is being planned.

Niacin contents within 10 per cent of the initial values found for canned pork luncheon meat at the end of 12 months' storage at temperatures of 7 to 37°C., Feaster *et al.* (1946), suggest that this vitamin is extremely stable. These observations are in agreement with those of Rice and Robinson (1944). A recent report, Moschette *et al.* (1947), indicates that the niacin values of canned peaches, tomatoes, and tomato juice are little affected during one year at temperatures of 10 to 27°C.

Stability of pantothenic acid in canned tomato juice, green lima beans, and whole kernel yellow corn was found, Guerrant *et al.* (1945), to be associated with storage temperature. At low temperature, the retention was approximately 85 per cent as compared to 45 per cent after one year at the high temperature of 43°C. Retention of this vitamin was quite good, Feaster *et al.* (1946), Rice *et al.* (1944), in pork luncheon meat during storage. However, decreases of the order of only 10 per cent were generally observed at high temperature (37°C.) for one year.

Information regarding the influence of storage on the newer members of the B complex in canned foods has not come to the writers' attention.

QUALITY

Published information regarding the influence of storage on the quality characteristics of canned food is rather limited. However, it is generally recognized that prolonged holding under adverse conditions is distinctly

detrimental to quality. In addition to temperature, the type of product, preparation and packing methods, and type of container are factors influencing quality retention in storage.

Color is one of the properties of canned foods affected by storage. Highly pigmented fruits, such as cherries, grape juice, Italian prunes, blueberries, and others are packed in enamel-lined cans to retard bleaching of the pigments by tin and its salts.

In conventional canning procedures, the chlorophyll in peas and other vegetables is decomposed, resulting in the loss of the characteristic green color. In a special canning procedure described by Blair and Ayres of this laboratory (1943), a large portion of the chlorophyll is retained in the canning process. However, in these peas, a gradual decline in chlorophyll content accompanies holding. Rate of color loss is a function of temperature, and storage at low temperature is desirable for maximum color retentions.

At normal temperatures of 21°C.(70°F.) or lower, changes in the color of most canned foods are not of great commercial significance. However, holding of canned foods at abnormally high temperature is accompanied by loss of the characteristic bright colors of the pigmented fruits and development of the dull and darkened shades in the light-colored products. Reactions between the proteins and carbohydrates of the type described by Cantor (1946) are probably associated with these changes.

The early literature on browning of orange juice was reviewed by Joslyn and Marsh (1935) who reported that the degree of browning was influenced by oxygen content. Subsequent studies, Boyd and Peterson (1945), Josephson *et al.* (1945), Lueck *et al.* (1941), Nolte *et al.* (1942), Riester *et al.* (1945), have established that keeping the oxygen content of citrus juices and other products at a minimum assists in retarding formation of undesirable color. Contact of the product with tin of the can tends to retard darkening. However, at present, it is generally agreed that oxidation is not the only factor influencing browning of citrus juices during storage and although plain tin cans retard, they do not completely prevent, browning under unfavorable conditions.

Flavor is also subject to change in storage. In the case of corn and asparagus, detectable differences in flavor were noted among samples held six months at 4, 16, 27, and 37°C.(40, 60, 80, and 98°F.), with samples stored at the lower temperatures possessing the more desirable taste characteristics. Flavor of tomato juice held in the stacks of a warehouse for four months was not noticeably different from that of samples stored at 4°C. for the same period. At the end of nine months, a slight difference in favor of the low temperature samples was noted in tomato juice stored at 4 and 21°C. However, flavor changes accompanying storage of most canned foods at 21°C. for one year are so slight they are difficult to detect.

On the other hand, the flavors of orange and other citrus juices are quite sensitive to storage. At the end of three months at 37°C., orange juice usually possesses a distinctly unpleasant flavor and aroma and may be entirely unacceptable. Peel oil, oxygen content, and type of container, Boyd *et al.* (1945), Riester *et al.* (1945), are factors influencing changes in flavor. Addition of certain antioxidants to this product prolongs the

induction period as measured by peroxide values of the extract, Nolte *et al.* (1942), but no correlation has been found between peroxide values and flavor. In fact, the basic causes for the early development of off-flavor in canned orange juice during storage have not, as yet, been fully established. One year at 21°C. results in some deterioration; hence, it is desirable to store citrus products at low temperatures.

Texture, an important quality factor, is influenced by storage conditions. Tomatoes, asparagus, and some other canned foods tend to become more firm, thus developing more desirable texture during short storage after canning. Addition of small quantities of a calcium salt to tomatoes at the time of packing is favorable to this firming process, Kertesz *et al.* (1940). However, storage beyond the initial short period does not appear to be favorable to texture of canned foods. Holding under reasonable conditions does not seriously affect the texture in normal marketing periods. At high temperatures, several canned foods tend to lose desirable firmness during prolonged holding.

TABLE 2

Influence of Storage on Firmness of Canned Shrimp

Examination	Penetrometer readings [1]
	1/10 mm.
Initial	63.1
After 6 mo. at room temperature	65.4
After 6 mo. at 98°F.	73.0
After 12 mo. at room temperature	70.6
After 12 mo. at 98°F.	75.6

[1]Average of readings for sixty shrimps of each variable.

Loss of firmness in canned shrimp during storage is of considerable economic importance. In studying methods for improving the texture of this product, it became obvious that hand-grading for firmness over a period of time does not permit accurate recording of the effects of storage. Working in this laboratory, J. K. Cameron adapted the "Precision Penetrometer" to the grading of shrimp for firmness, which eliminates certain of the uncertainties of hand-grading.

In making the test, a well-defined segment, extending across the entire side of the shrimp meat is selected as the point at which to measure firmness. With the specimen lying on its side, a sharp-pointed cone of known weight is lowered until it just touches the specimen to be tested. The cone is released by a trigger mechanism and allowed to penetrate the material. At the end of 10 seconds, the depth of penetration, in 0.1 millimeters, is read from a dial and recorded as a measure of firmness.

Firmness of individual shrimps which had been subjected to a definite treatment was found to vary over a considerable range. Statistical analysis of penetrometer readings indicates that examination of thirty shrimps yields a fairly reliable average. The influence of storage on texture of canned shrimp is indicated (Table 2). These data suggest a definite change in the firmness of this product during storage. Using this analytical procedure, it was found that the prepacking treatments, or addition of small

amounts of calcium chloride to the liquid packing medium, influence the initial firmness as well as the change during storage.

High temperature has been shown to be detrimental to the quality of canned foods. However, the possible influence of low or freezing temperature should also be considered. Periods of severely cold weather may result in accidental freezing of some canned foods in transit or in unheated warehouses. Considering the problem of accidental freezing from the viewpoint of utilization of canned food by the ultimate consumer, Smith and Smith (1944) observed that color and flavor are not damaged. However, the consistency of some products was adversely affected. One of the more noticeable changes was the separation of liquid in foods containing cooked starch. On reheating for serving, the normal consistency of frozen samples was restored.

Although freezing does not prevent the utilization of canned foods, it may be a factor influencing the economic value of some products. Braun and Hoynak of this laboratory observed the following effects of multiple freezings and thawings on typical canned foods:

Asparagus: Severe breakdown of the heads, loss of tenderness of the stalks, and lower drained weights.

Green Beans: Excessive split pods and loose skins.

Cream Style Corn: "Weeping," when examined at room temperature—mealy texture.

Whole Kernel Corn: Breakdown in cell structure and "rubbery" texture.

Early June Peas: Slight mealiness in the cotyledon and increase in the number of splits.

Spinach: Breakdown in texture, loss of crispness, development of "gummy" texture, with decreases in drained weight.

Tomatoes: Loss of fluids from the cells, with loss of drained weights. This effect is illustrated by the following figures: Drained weights before freezing, 13.4 ounces; drained weights after one freezing, 9.4 ounces; drained weights after multiple freezings, 8.7 ounces.

Tomato Juice: No significant change.

Blackberries: Very slight softening.

Clingstone Peaches: No significant change.

These observations demonstrate that freezing of many canned foods while in trade channels may result in economic losses due to downgrading. For this reason precautions should be taken against the accidental freezing of canned foods that might be adversely affected.

SUMMARY

Canned foods are to be classed among the more stable categories of foodstuffs. However, it is well established that they are subject to some chemical and physical changes during storage. Of the vitamins, ascorbic acid and thiamin are affected to the greatest extent. In the first month after packing, any atmospheric oxygen entrapped in the can is consumed. During this period, ascorbic acid retention may be influenced by the type of product, amount of oxygen, and type of container (plain tin cans being preferable to glass containers or enamel-lined cans for some foods). Beyond the initial short storage periods, temperature is the most important

factor from the viewpoint of ascorbic acid and thiamin survival. At 37 to 43°C. temperatures, at which canned foods may be held for very limited periods of time in civilian distribution or for longer periods in certain tropical military operations, vitamin destruction proceeds at a measurable rate. At 21°C., which may be near the average of year-round commercial storage conditions, ascorbic acid and thiamin retentions during one year appear to be in the region of 75 to 90 per cent. Carotene, vitamin D, niacin, riboflavin, and pantothenic acid retentions are reported to be even higher, in the limited number of studies available.

The quality of canned foods as evidenced by color, flavor, aroma, and texture is also influenced by storage conditions. At moderate temperatures (21°C. or below) storage of most canned foods for one year does not appear to have any marked influence on quality. However, in the case of some few of the more susceptible products, changes in flavor under usual storage conditions may be of commercial importance. For such foods, refrigerated storage is indicated. Freezing has an adverse effect on the texture of some canned vegetables which results in downgrading. However, freezing does not affect the utilization of these products.

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THIAMIN, RIBOFLAVIN, AND NIACIN CONTENT OF WALNUTS

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Tables of food composition must obviously now include accurate values for vitamin content. How these values are to be arrived at remains to some extent in the realm of controversy. Chemical, physical, microbiological, and biological means of assay have been developed rapidly in recent years, but few of these methods are sufficiently specific and uncomplicated by the action of interfering substances to be acceptable without corroborating evidence. Each procedure has been developed by specialists not always experienced with the method used for comparison. Furthermore, the errors in the latter may mask those in the former. Establishment of criteria for vitamin analytical procedures has received much attention in collaborative studies, but has not yet been satisfactorily accomplished.

In most comparisons of the results of chemical, physical, and microbiological procedures with those of the biological assay for vitamins, a discrepancy is found, particularly in the case of low potency samples. Yet, most foods are of low potency and their accurate analysis is of primary importance in human nutrition. Each food presents a separate problem since each has different concomitant substances which may operate differently in the various methods of analysis.

The study of walnuts was undertaken with these difficulties in mind and an effort was made to validate each of the methods used, by recovery experiments and by comparison with other methods.

REVIEW OF METHODS

Thiamin. Weight maintenance, growth-rate, bradycardia, and the cure or prevention of polyneuritis in rats and birds are among the bio-assay techniques for thiamin determination. The yeast growth method which Schultz, Atkin, and Frey (1942) improved with sulfite cleavage of thiamin in the blank and the use of the fermentometer apparatus has often been used for comparison with the chemical methods.

The most sensitive and widely used procedure for the chemical determination of thiamin is the measurement of the thiochrome fluorescence produced upon oxidation of thiamin. Improvements on this method have been suggested by Hennessy and Cerecedo (1939) who used activated "Decalso" to adsorb thiamin preferentially and thus eliminate interfering substances from the eluate. Others have found the adsorption step to be unnecessary or undesirable in certain instances. Wang and Harris (1939) claimed incomplete elution of the vitamin in some cases and the simultaneous concentration of interfering substances by adsorption. To overcome this difficulty they washed the samples with isobutanol before oxidation and also washed the thiochrome-containing alcohol with water.

The most critical step in the determination is the oxidation. Watson (1946) reviewed the procedures of various workers and presented a comprehensive study of factors influencing the oxidation of thiamin to thiochrome. The order in which the reagents were added and their concentration were found to be important.

Release of thiamin from its pyrophosphoric-acid ester and from carbohydrate or protein combinations is necessary in non-biologic assays. Methods of hydrolysis suggested by Hennessy and Cerecedo (1939), Wang and Harris (1939), Conner and Straub (1941), and others include the action of many enzyme preparations on various foods. Takadiastase and clarase seemed most efficient, probably because of phosphatase activity. The choice for any given food has to be the result of experiment.

The main considerations, then, in adapting the thiochrome method to different materials are—(1) extraction, with or without enzyme digestion; (2) purification by adsorption or washing with isobutanol as needed; (3) oxidation to thiochrome and subsequent measurement of fluorescence.

Riboflavin: The rat-growth method, microbiological assay, visual fluorometry, and photoelectric fluorometry are satisfactory methods for riboflavin analysis. Comparison of analyses made by all these methods on the same samples have occasionally been reported, but usually the samples studied were of high vitamin concentration. More often only the microbiological and fluorometric methods have been compared. The chief difficulties in both chemical and microbiological analyses are inefficiency of extraction of the vitamin and simultaneous extraction of interfering substances which may give erroneously high or low potencies for the extracts.

Snell and Strong (1939) proposed the microbiological method which has been used in many laboratories. Extraction is usually accomplished by autoclaving or steaming in 0.1 N acid, by digestion with an enzyme, or by a combination of both procedures. Ether extraction has been recommended for removal of fat from samples which contain much lipoidal material, since fatty acids and other fat derivatives may produce serious "drifts" in the values obtained when different aliquots of the same extract are used, Strong and Carpenter (1942). However, filtration at pH 4.5 was shown to remove interfering substances better than ether treatment, Wegner *et al.* (1942).

The fluorometric method is complicated by the presence in many samples of pigments and fluorescent materials other than riboflavin. These may be removed by preliminary reduction with hydrosulfite and stannous chloride, Hodson and Norris (1939) or oxidation with potassium permanganate. Conner and Straub (1941a) advocated adsorption of the riboflavin on clays, Supersorb or Florisil, followed by elution with pyridine-acetic acid and oxidation. The adsorption, however, has been found to vary with the Florisil lot and omission of this step has been recommended by Mackinney and Sugihara (1942), McLaren, Cover, and Pearson (1944), and Rubin *et al.* (1945).

In nearly all these studies involving the fluorometric method, accuracy and sensitivity of the procedures have been judged by the agreement with the microbiological method.

Niacin: Biological, chemical, and microbiological assays for niacin have been in use for several years. The bio-assay with dogs fed the blacktongue-producing diet has been little used since blacktongue has been recognized as due to a multiple-deficiency. The chemical method depends upon the reaction of unmethylated pyridine derivatives with cyanogen bromide and aniline to form a colored compound. Dann and Handler (1941), among others, have reviewed the difficulties involved in the application of this reaction to the determination of niacin in foods and biological materials.

The microbiological method for niacin appears to be specific and sensitive and is in general use. The procedure of Snell and Wright (1941), modified by Krehl, Strong, and Elvehjem (1943), using *Lactobacillus arabinosus*, has been followed in many laboratories with satisfactory results. The recovery of added niacin and acceptable replication have been used to check the accuracy and specificity of the method. There have been only a few comparisons with either the response of the blacktongue dog or the chemical method, Daniel and Norris (1945).

VITAMIN CONTENT OF WALNUTS

Only a few quantitative studies have been reported on the vitamin content of walnuts. Ascorbic acid was reported in concentration of more than 1,000 mg. per cent in the green hulls, 350 to 390 mg. per cent in the leaves of *Juglans regia,* and 1,550 to 1,650 mg. per cent in the green fruit of *Juglans nigra,* Hennig and Oshke (1940). This was confirmed by Pyke, Melville, and Sarson (1942) who recommended unripe walnut pickles and confections as a rich source of vitamin C. The dry, mature nuts retain only a trace of this vitamin.

The kernels of English walnuts were reported by Mignon (1923) to contain vitamin A activity which was extracted by hot alcohol but was not demonstrable in the cold-pressed or ether-extracted oil.

De Caro and Franceschini (1939) found the equivalent of 130 IU vitamin A per 100 grams of mature, dried walnut kernels using rat assay with beta-carotene standards. Booher and Marsh (1941) reported about 40 IU per cent by bio-assay.

Thiamin was reported present in walnuts to the extent of 0.28 mg. per cent by De Caro and Franceschini (1939) using a rat-survival method. Employing the rat-growth procedure, Booher and Hartzler (1939) found 0.34 mg. per cent thiamin in English walnuts, and Baker and Wright (1935), using the bradycardia method, found 0.45 mg. per cent.

Riboflavin and niacin analyses of walnuts have apparently not been published.

EXPERIMENTAL PROCEDURE

The samples of *J. regia,* used in this study, were grown in three districts in California and were of three varieties: Placentia, Franquette, and Payne. The samples were collected from the same groves[1] for four successive years and were selected from the best grade of nuts. They were stored both shelled and unshelled at 2°C.(35°F.).

[1] The California Walnut Growers' Association, Los Angeles, California, and Mr. Arthur W. Christie kindly secured, stored, and shipped all the samples.

Proximate analysis of the nuts indicated 14 to 16 per cent protein ($N \times 6.25$), 64 to 67 per cent fat, 1.8 per cent ash, and three to five per cent moisture. There were no significant variations among the three varieties or the four crops.

A study of the thiochrome and bio-assay methods for thiamin and of the fluorometric and microbiological methods for riboflavin was undertaken. Niacin determinations were by microbiological means. The validity of the chemical and microbiological procedures was judged by recovery of known amounts of the vitamins and by the reproducibility of results at the same and at different levels of concentration.

Thiochrome Method for Thiamin Determination: The thiochrome method was adapted to the nut analysis as the result of a series of experiments to determine the effects of (1) the nut oil, (2) the efficiency of the acid extraction of the thiamin with or without heating, (3) the choice of enzyme and optimum digestion period for the release of thiamin, (4) efficacies of adsorption and isobutanol washing for purification, and (5) the effect of dilution on the efficiency of the isobutanol washing.

It was found in the preliminary experiments that removal of the fat by pressure or ether extraction had no effect on the thiamin values or on thiamin recovery. The fat removal step was therefore omitted. It was also found that the usual hot water bath treatment of the nut meal in dilute sulfuric acid before enzyme digestion produced a slow-filtering, gelatinous mass. By omitting the heating, a rapid-filtering product with improved thiamin recovery was obtained.

Adsorption on Decalso columns with elution of the thiamin by hot KCl solution was first used to attain separation from interfering substances. Recovery of added thiamin was used in this and in all later comparisons as the criterion of efficiency. Recoveries were variable and excessive (see Table 1, column 1). The chief factor appeared to be the dilution used in the fortified extracts. This changed the color of the extracts and might have affected the readings if the pigment had not been adequately removed during adsorption. Since the eluates were colored, and since the isobutanol might have extracted an interfering substance along with the thiochrome, a preliminary isobutanol wash seemed desirable. A non-thiamin reductant might also account for lower thiochrome readings in the less dilute extract, if the amount of ferricyanide used were insufficient to oxidize both thiamin and other reductants.

Recovery of thiamin added to the walnuts was then determined after various treatments for the removal of interfering substances. These results are shown (Table 1). It is evident that direct oxidation of the extracts gave low recovery, adsorption on Decalso and elution with KCl before oxidation gave high and variable results, and pre-oxidation washing with isobutanol gave low recoveries which improved with the use of a larger amount of ferricyanide; but the dilution factor was still operative.

The isobutanol wash was adopted tentatively as the method of purification to study the effect of dilution. Aliquots of walnut extracts, with and without added thiamin, were purified by isobutanol without further dilution, 1:20 of sample to extract, and after dilution of 1:40. To one of

duplicate aliquots of each extract in the reaction vessel was added 0.2 μg. thiamin. The undiluted aliquots yielded 28 and 20 per cent and the diluted 64 and 72 per cent recoveries from the original extracts. Of the 0.2 μg. thiamin added to the 1:20 aliquots in the reaction vessels 25 and 35 per cent were recovered, while 60 and 55 per cent were recovered from the 1:40 extract.

Further dilution of the extracts increased the recoveries significantly. The 1:60 dilution (one-gm. sample in 60 ml. extract) prior to oxidation gave reproducible results and quantitative recoveries (Table 1, column 3).

TABLE 1

Recovery of Thiamin Added to Walnut Extracts as Affected by Method of Purification and Dilution

Sample	1	2	3	4
	Adsorption on Decalso	No purification	Isobutanol wash	Dilution
	μg./gm.	μg./gm.	μg./gm.	
Walnuts	3.2, 1.4,[1] 3.3	0.2	0.5,[2] 1.2, 1.4	1:20
			2.8	1:40
			2.9	1:60
			3.4	1:80
Walnuts plus 3 μg. thiamin per gm.	7.2, 5.8,[1] 6.7	1.4	2.1,[2] 3.3	1:40
Walnuts plus 1 μg. thiamin per gm.	.....	.....	1.8	1:20
			3.4	1:40
			3.9	1:60
			4.2	1:80
Thiamin recovery, pct.	131, 147, 113	40	52, 70, 40	1:20
			60	1:40
			100	1:60
			80	1:80

[1] Digested at 40 to 45°C.(104 to 113°F.) at pH 4.5 with 0.5 per cent clarase for six hours. All others were similarly digested for 17 hours. [2] Oxidized with one mg. ferricyanide. All others were oxidized with two mg. ferricyanide.

Finally, clarase and papain, singly and together, were compared for effectiveness in release of thiamin in the extracts. Recoveries of one μg. thiamin added per one gm. of nuts was the basis for judging the activity of the three enzyme preparations. The digestion was carried out at 37°C. (99°F.) for 17 hours using: (1) 0.5 per cent papain, (2) 0.5 per cent clarase, or (3) 0.5 per cent papain plus 0.5 per cent clarase in media of pH 4.5. The unfortified extracts produced by papain, clarase, and papain-clarase digestion yielded values of 2.1, 2.6, and 2.6 μg. thiamin, respectively, per gram sample. Values for the corresponding fortified extracts were 2.5, 3.6, and 3.4 μg. per gram sample. Hence, the recoveries of the added thiamin were 40, 100, and 80 per cent. The larger yields from the nuts, as well as the excellent recovery, pointed to the use of clarase.

The method of extraction finally adopted was as follows: Walnut kernels were disintegrated in a Waring blendor and five-gram portions put into 60 ml. 0.1 N sulfuric acid and the acidity adjusted to pH 4.5 with

sodium acetate. The samples were allowed to digest overnight (about 17 hours) at 37°C. with 0.35 gm. of clarase enzyme preparation in pH 4.5 acetate buffer. The extract was filtered, the residue washed with distilled water, the filtrate neutralized and refiltered prior to making it to volume (100 ml.).

In the 1946 determination on the walnut samples, the oxidation was conducted on an aliquot of the original extract which had been diluted one to three and washed with isobutanol, 10 ml. of isobutanol to 15 ml. of the diluted extract. A five-ml. aliquot of the washed extract was pipetted into each of two reaction vessels. Three ml. of a 15 per cent sodium hydroxide solution were delivered into each vessel and one ml. of 0.2 per cent potassium ferricyanide added to one vessel immediately after the alkali. The vessels were agitated, 15 ml. of purified isobutanol pipetted into each, and then shaken vigorously for one minute. After the separation of the phases by centrifugation, the aqueous layer was discarded, the alcohol dried with anhydrous sodium sulfate, decanted into a cuvette, and its fluorescence read. The fluorescence reading of the unoxidized blank was subtracted from the reading of the oxidized aliquot and the net value compared with that of the standard thiamin solution. Correction was made for the enzyme blank. Results obtained by this method are shown (Table 2).

TABLE 2

Thiamin Content of Walnuts Determined by Thiochrome Method, Purification by Isobutanol Wash (1946)

Variety	Crop Year	Storage		Thiamin	Chemical / Biological ×100
		Form	Mo.		
				mg./100 gm.	
Franquette	1942	Shelled	42	0.19	76
	1943	In shell	30	0.20	63
	1944	In shell	18	0.21	.....
		Shelled	18	0.25	.....
	1945	In shell	6	0.23	.....
		Shelled	6	0.22[1]	.....
Payne	1942	Shelled	42	0.28	76
	1943	In shell	30	0.22	73
		Shelled	30	0.24	69
	1944	In shell	18	0.32	.....
		Shelled	18	0.32	.....
	1945	In shell	6	0.32[1]	.....
		Shelled	6	0.33[1]	.....
Placentia	1942	Shelled	42	0.28	88
	1943	In shell	30	0.26	79
	1944	Shelled	18	0.30	88
	1945	Shelled			
		Light	6	0.29[1]	62
		Amber	6	0.28[1]	54
		Dark	6	0.22[1]	65
		In shell			
		Amber	6	0.29[1]	.....

[1] Three determinations. Six determinations were made on all others. The separate determinations were not made simultaneously.

In the earlier series (1943) of determinations the same extraction procedure was followed, but purification was effected by adsorption of the thiamin on Decalso, following the method described by Conner and Straub (1941). These results are noted (Table 3).[2]

TABLE 3

Thiamin Content of Walnuts Determined by Thiochrome Method, Purification by Adsorption on Decalso (1943)

Variety	Crop Year	Storage		Thiamin
		Form	Mo.	
				mg./100 gm.
Franquette	1942	In shell	15	0.27
		Shelled	15	0.27
	1943	Shelled	3	0.28
Payne	1942	In shell	15	0.36
		Shelled	15	0.35
	1943	Shelled	3	0.32
Placentia	1942	In shell	15	0.41
		Shelled	15	0.34
	1943	Shelled	3	0.29

Bio-assay Method for Thiamin Determination: Twenty-one- to twenty-seven-day-old rats of the Long-Evans strain weighing 38 to 42 grams were placed at weaning on a vitamin-deficient basal ration supplemented with two ml. of sulfited rice bran extract[3] with added crystalline vitamins and six to eight drops of a vitamin A-D-E oil solution given three times a week in castor cups. The composition of the basal diet was: Powdered sucrose 66.5, vitamin-free casein 22.0, fat[4] 9.0, and salts, Hubbell *et al.* (1937), 2.5. The vitamin supplement provided 20 μg. each of pyridoxine and riboflavin, 100 μg. each of calcium pantothenate and para-amino benzoic acid, and 50 mg. of choline per rat per day. In addition, whatever biotin, folic acid, and unknowns that may have been required were presumably furnished by the rice bran extract. The A-D-E oil was a Wesson oil solution of shark-liver oil, irradiated ergosterol, and mixed tocopherols from distilled natural vegetable oils to furnish 1,000 IU vitamin A, 100 IU vitamin D, and 3 mg. vitamin E per rat per week when administered in three doses of six to eight drops.

The young rats were divided into assay groups composed of eight to 10 animals balanced as to sex, litter, and size when the weights became stationary. They were then given an additional supplement of either a standard thiamin solution or a nut dose. Thiamin hydrochloride was given so as to supply a daily intake equivalent to two, four, six, or eight micrograms. There was also a negative control group which was kept on the thiamin-deficient diet. The nuts were fed three times a week so that the intake was one gm. chopped kernels per day.

[2] These determinations were made by Lelia C. Rawlins.

[3] A rice bran concentrate manufactured by Galen Company of Berkeley, California.

[4] Primex, a hydrogenated cottonseed oil product.

The thiamin standard groups were continued on the basal ration consumed during depletion, but those given the nut supplements were given a basal diet modified to compensate for the high fat content of the nuts. The percentage composition of this diet was: Powdered sucrose 73.0, vitamin-free casein 22.0, fat 2.8, salts, Hubbell *et al.* (1937), 2.2. A record of the basal ration consumed by each animal was kept and the total amount for each group tabulated.

The average weight-gains for four weeks for the standard groups were plotted against their daily thiamin intakes to obtain the standard growth-rate curve. On this curve the thiamin content of the walnuts could be estimated (Table 4).

TABLE 4

Thiamin Content of Walnuts Determined by Bio-assay

Variety	Crop year	Storage		Thiamin	Thiamin retention in storage	
		Form	Mo.		Mo.	Pct.
				mg./ 100 gm.		
Franquette	1942	In shell	9[1]	0.43	.....	.....
		Shelled	9[1]	0.32	.....	.....
		Shelled	42	0.25	33	78
	1943	In shell	30	0.32	.....	.....
Payne	1942	In shell	9[1]	0.43	.....	.....
		Shelled	9[1]	0.48	.....	.....
		Shelled	42	0.37	33	77
	1943	In shell	42	0.30	.....	.....
		Shelled	42	0.35	.....	.....
Placentia	1942	In shell	9[1]	0.47	.....	.....
		Shelled	9[1]	0.40	.....	.....
		Shelled	42	0.32	33	80
	1943	In shell	30	0.33	.....	.....
	1944	Shelled	6[1]	0.40	.....	.....
		Shelled	18	0.34	12	85
	1945	Shelled	6	.....	.....	.....
		Light	.....	0.47	.....	.....
		Amber	.....	0.52	.....	.....
		Dark	.....	0.34	.....	.....

[1]Assays conducted by Mary Grundy.

Fluorometric Method for the Determination of Riboflavin: The walnut extracts used in the thiochrome determinations were found to be suitable for the riboflavin and niacin determinations, also. Again, the omission of the initial heating step was advantageous, since both riboflavin recovery and apparent content of riboflavin in the walnut extracts were larger when the samples were not pre-steamed. The Mackinney and Sugihara (1942) modification of the Conner and Straub (1941a) procedure was employed with further changes. All operations were carried out in diffuse light. Twenty-five ml. of the original extract were delivered by pipette into a fifty-ml. volumetric flask and the solution made to volume. Duplicate 10-ml. aliquots of the diluted extracts were pipetted into fifty-ml. beakers containing five ml. of two per cent acetic acid. After all extract

aliquots were taken, one ml. of four per cent $KMnO_4$ was added to each by means of a graduated one-ml. pipette and the solutions mixed by swirling. This was followed by the addition of one ml. of three per cent hydrogen peroxide and further mixing. One such preparation of each extract was kept cold while being photolyzed with a 250-watt lamp for four to five hours. After photolysis, the extracts were transferred to cuvettes for reading in the Coleman Model 12 photofluorometer. The cuvettes were arranged in two parallel rows and the photolyzed blanks put behind those not photolyzed and read immediately after the unphotolyzed aliquots. A standard representing 0.5 μg. riboflavin in 10 ml. was treated similarly and read first.

TABLE 5

Effect of Ether Extraction on Riboflavin and Niacin Values of Placentia Walnuts Determined by the Microbiological Method

Extracts	Riboflavin (μg./gm.)		Niacin (μg./gm.)	
	Ether-extracted	Not ether-extracted	Ether-extracted	Not ether-extracted
Autoclaved 20 min. at 15 lb.				
Whole kernels	1.0	1.0	.....	.....
Skins	1.6	1.9	16.0	15.3
Meats	1.4	1.7	12.4	13.0
Steamed 20 min., clarase digestion 2 hr.				
Whole kernels	1.5	1.6	.....	.....
Skins	1.3	1.4	14.3	15.0
Meats	1.4	1.5	11.3	11.3
Clarase digestion 2 hr.				
Whole kernels	1.6	1.7	.....	.....
Skins	1.6	1.7	13.3	15.0
Meats	1.8	1.8	11.8	11.1

The removal of interfering substances by means of sodium hydrosulfite recommended by Hodson and Norris (1939) and Andrews, Boyd, and Terry (1942) was not feasible, since this procedure bleached the extract and gave high blanks. The reverse was true when a drop of saturated sodium hydroxide was used as recommended by McLaren *et al.* (1944) because the alkali darkened the extract. Recovery of added riboflavin by the method adopted was 98 per cent.

Microbiological Method for the Determination of Riboflavin: The method of Snell and Strong (1939) presented as the tentative USP assay (1944) was followed with slight modifications. The medium was diluted so that nine ml. were added to each tube. Three levels of each extract were assayed, the aliquots being 0.6, 0.8, and 1.0 ml., respectively. The riboflavin content of each tube was determined from the standard curve, made at the time of each assay. The points on the curve, determined in duplicate, were 0.0, 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, and 0.40 μg. riboflavin per tube. Inoculation with *Lactobacillus casei* was from a sterile syringe and the samples were incubated at 37°C.(99°F.) for about 65 hours. Titration with 0.1 N NaOH, using bromthymol blue as the internal indicator, was carried out in the incubation tubes, mixing being accom-

plished by bubbling air through the solution. The amounts of riboflavin at the three assay levels were converted to the amount in one ml., the average of the three values determined, and thence the riboflavin content per gram of walnuts. Added riboflavin was recovered to the extent of 90 per cent.

Since the high fat content of the nuts might interfere with the analyses, an aliquot of several extracts was shaken with ether in a separatory funnel prior to incubation with the microorganism. Results presented (Table 5) show no advantage afforded by the ether treatment.

Microbiological Determination of Niacin: Essentially the tentative USP XII assay (1944) was used. Ten ml. of medium were added to the tubes containing 0.2, 0.6, and 0.9 ml. of each extract (1:40 dilution) to be assayed. The points on the standard curve and the calculations were made as described for the riboflavin determination. Results of recovery tests indicated 99 per cent efficiency. Removal of the nut oil from the extracts was found to have no appreciable effect upon the niacin determination (Table 5), and consequently this step was not included.

RESULTS AND DISCUSSION

The Thiamin Analyses: Thiamin values obtained by the thiochrome procedure were larger when the adsorption purification method was used in 1943 (Table 3) than when isobutanol washing was substituted in 1946 (Table 3). Recovery experiments (Table 1) indicated the same tendencies. If the results of the two series can be compared it would seem that the nuts retained 70 to 80 per cent of the thiamin after 27 months in cool storage. The 1944 and 1945 samples analyzed by the non-adsorption technique after 18 or six months' storage, in most cases were close to those found by the adsorption method after 15 or three months' storage of the 1942 and 1943 samples, respectively. The nuts stored in and out of the shells were alike in thiamin value. The Franquette variety had 20 to 30 per cent less thiamin than comparable samples of the Payne and Placentia varieties.

The bio-assays (Table 4) allowed comparisons of samples stored 33 or 12 months. The 77 per cent thiamin retention in these samples was similar to that of 1942 and 1943 samples stored for 27 months.

The caloric intake per gram of gain was found to decrease rapidly as the thiamin level was raised. Fifty-four calories were used per gram gain in the negative group and a constant value of 15 calories per gram gain was reached when four, six, and eight μg. of thiamin were taken in per day. The four-μg.-day level represented the most efficient utilization of thiamin and was also the level used in the walnut-fed groups. Variability of gain was the same in all groups, the standard deviation being 10 ± 3 grams, and a probable error of the means being 2.3 ± 0.7 grams.

Storage in or out of shells had no significant effect on thiamin retention as shown also by bio-assay. The Franquettes allowed less growth than the other two varieties. Three samples of 1945 Placentias, selected as to color, were found to vary in thiamin value, the dark-skinned kernels having 22 to 28 per cent less than the light or amber as shown by both methods of assay.

In all comparable cases the rat-growth assay yielded higher thiamin values than the chemical procedure, with less discrepancy in the fresher samples. This may indicate the presence of some fluorescent substance extracted with the thiamin, quenched by the oxidative process, yet exerting its fluorescence in the unoxidized aliquot, thus producing high blanks. This theory is supported by the fact that dilution increased thiamin recoveries. In the course of aging, the interfering substance might be decomposed.

TABLE 6

Riboflavin and Niacin Content of Walnuts

Variety	Crop Year	Riboflavin				Niacin	
		Chemical (mg./100 gm.)		Microbiol. (mg./100 gm.)		Microbiol. (mg./100 gm.)	
		In shell	Not in shell	In shell	Not in shell	In shell	Not in shell
Franquette	1942	.....	0.11	.....	0.09	.....	0.55
	1943	0.10	.....	0.09	.....	0.58	.....
	1944	0.11	0.16	0.09	0.13	0.60	0.68
	1945	0.12	0.12	0.13	0.08	0.51	0.60
Payne	1942	.....	0.17	.....	0.14	.....	0.86
	1943	0.12	0.11	0.09	0.09	0.57	0.56
	1944	0.18	0.18	0.10	0.12	0.81	0.98
	1945	0.16	0.18	0.12	0.13	0.67	0.74
Placentia	1942	.....	0.15	.....	0.12	.....	0.91
	1943	0.17	.....	0.13	.....	0.90	.....
	1944	.....	0.17	.....	0.12	.....	0.94
	1945	0.18A[1]	0.17A	0.13A	0.12A	0.66A	0.69A
		.....	0.20L	.....	0.18L	.....	0.86L
		.....	0.12D	.....	0.12D	.....	0.87D

[1] L, A, and D designate light-, amber-, and dark-colored kernels.

Recovery of added thiamin near 100 per cent is not always a sound criterion for the validity of the method. In this case the biological assays which were carefully controlled indicated significantly larger values in both series of measurements. Loss or destruction of thiamin during analysis may be detected through recovery experiments, but false values by chemical determination due to interfering substances in the test material may still result. The rat-growth procedure, on the other hand, is likely to indicate the true physiological worth of the food and in low-potency food samples appears to have about the same degree of precision as the chemical method.

Riboflavin and Niacin Analyses: Average values of three or six assays on the various walnut samples for riboflavin and niacin, all carried out in the early summer of 1946, are presented (Table 6).

There seemed to be little difference in retention of riboflavin in the kernels stored in or out of the shells, and little difference between crop years. This indicated good vitamin retention during storage for from six months to three years under these conditions. The niacin content of the kernels not stored in the shells was 11 to 13 per cent higher than like samples stored in the shells. The dark-colored Placentias were 30 to 40

per cent lower in riboflavin than the light- or amber-colored ones, but had the same niacin content as the light kernels.

A comparison of values obtained by the different methods revealed that the microbiological assays for riboflavin gave results about 20 per cent lower than the fluorometric method. The discrepancy is difficult to explain, unless some fluorescent substance such as might be interfering in the thiamin assay is also operative here, this time to give high results. Recoveries of added riboflavin and the reproducibility of results were somewhat better in the fluorometric than in the microbiological assays. Range of values obtained by both methods must probably be accepted for the present.

Overall average values for thiamin, riboflavin, and niacin of Placentia, Franquette, and Payne varieties of walnuts are shown (Table 7). The Franquettes had 20 to 25 per cent lower content of each of the three vitamins than the Placentia or Payne varieties, which were about equal in potency.

TABLE 7

Average Thiamin, Riboflavin, and Niacin Values of Placentia, Franquette, and Payne Walnuts[1]

Variety	Thiamin		Riboflavin		Niacin
	Chem.	Biol.	Chem.	Microbiol.	Microbiol.
Placentia	0.29	0.40	0.165	0.125	0.81
Franquette	0.24	0.33	0.120	0.100	0.58
Payne	0.31	0.39	0.155	0.110	0.73

[1] In mg. per 100 gm. kernels.

The Food and Drug Administration Laboratory[5] reported to the Quartermaster of the United States Army analyses on walnut samples similar in origin to those used in our experiments. Thiamin determinations on Franquette, Payne, and Placentia nuts of the 1943 and 1944 crops, made by the yeast fermentation method, Schultz *et al.* (1942), were found to be 0.43, 0.45, 0.43 and 0.62, 0.52 and 0.58 per cent, respectively. By thiochrome methods only one-third of these values were obtained, but bio-assay results agreed with those obtained by the fermentometer. Modification of the thiochrome procedure by removal of the fat brought these values to from 75 to 80 per cent of the fermentometer results. This is in good agreement with the corresponding comparison of the biological and chemical methods made in our laboratory.

Riboflavin tests made by the Food and Drug Administration group by the microbiological method agreed closely with ours obtained in the same manner, but the niacin analysis by the microbiological procedure resulted in values approximately 30 to 80 per cent greater than those reported in this paper. No explanation for this difference is available.

SUMMARY

A modification of the thiochrome method of determination of thiamin was applied to the analysis of walnuts of the Placentia, Franquette, and Payne varieties of four successive crops and was found to yield satisfac-

[5] Personal communication from E. M. Nelson.

tory recoveries of added thiamin, as well as good replication of results. The rat-growth assay was also found to yield reproducible values with the walnut samples, but these values were consistently 20 to 30 per cent larger than those obtained by the thiochrome procedure. The discrepancy may be due to the presence in nuts of oxidizable fluorescent substances destroyed by oxidation, with resultant high blanks and low thiochrome readings. Relatively little loss of thiamin occurred in storage up to 42 months and there was no difference in loss due to storage in or out of the shells. The Placentia, Payne, and Franquette walnuts contained 0.40, 0.39 and 0.33 mg. per cent thiamin.

The riboflavin content of these walnut samples determined by the microbiological method was usually found to be about 20 per cent less than appeared present when the fluorometric method was used. There was little loss of riboflavin in storage, up to 42 months, whether the kernels were stored in or out of the shells. The Placentia, Payne, and Franquette nuts contained 0.13 to 0.16, 0.11 to 0.16, and 0.10 to 0.12 mg. per cent riboflavin.

Niacin, determined by the microbiological method, was also retained well in storage, whether the kernels were in or out of the shells. The Placentia, Payne, and Franquette nuts contained 0.81, 0.73, and 0.58 mg. per cent niacin.

Comparable values were found in the nuts of the four crops, but in all cases the Franquette variety appeared to contain 20 to 30 per cent less of each of the three vitamins than did the other two varieties, which were alike in value. Dark-colored kernels of the 1945 Placentias contained 25 to 30 per cent less of thiamin and riboflavin than did the comparable light- and amber-colored ones.

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BEHAVIOR OF SULPHUR DIOXIDE IN CONCENTRATED ORANGE JUICE

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Sulphur dioxide, commonly used to preserve fruit juices, is much more effective for dilute than for concentrated juices. It has been suggested that this is because, in concentrated juices, only a fraction of the added sulphur dioxide remains in the free state, and only this fraction is effective in preventing the growth of microorganisms, Downer (1943), Ingram (1947c). Since the classic researches of Kerp it has been realized that if SO_2 is added to a medium containing sugars, notably glucose, a proportion of the SO_2 added forms bisulphite compounds with the sugar and only the remainder appears in the free state. Data presented here confirm that this is the case in concentrated orange juice, and they illustrate the way in which the balance between combined and free fractions varies with circumstances.

In pure solutions, Kerp (1904) found that the equilibrium glucose + $SO_2 \rightleftarrows$ glucose · bisulphite was reversible, so that the proportion between free and combined SO_2 should depend on the concentration of sugar and be maintained by readjustment of the equilibrium if the total quantity of SO_2 is changed. With large concentrations of sugar, as in concentrated juices, the free SO_2 is only a minor fraction of the total; but as there is good evidence that only this is effective in preventing the growth of microorganisms in the juice, it is evident that the behavior of this free fraction is the important subject. Nevertheless, since any changes in the total quantity of SO_2 will be reflected in a corresponding change in the free fraction, it is necessary first to consider changes in the total before the changes in the free fraction can be properly understood.

METHODS

Throughout this work, except where otherwise stated, we have used orange juice of United States manufacture, concentrated to 65° Brix, as delivered canned in Britain. Quantities of sulphur dioxide are quoted as parts per million w/w.

The total SO_2 has been determined by distillation into H_2O_2, following a modification, see Morris (1947), of the methods given by Monier-Williams (1927). This procedure is straightforward, and relatively accurate.

The free SO_2 is titratable by iodine, according to a procedure suggested by Downer (1943), which is improved by the use of an electrometric indicator, Ingram (1947a). The estimation is, however, difficult to make, because removal of the free SO_2 by titration leads to the production of more by breakdown of the bisulphite compounds, and occasionally erroneous results may be obtained with overheated juices, Ingram (1947b). Nevertheless, this procedure has been used in the present work, for want of better.

The free SO_2 estimated in this way includes gaseous SO_2, molecular H_2SO_3, and sulphite-bisulphite ions. It is believed, Ingram (1947c), that the total so measured is an index of the actually germicidal fractions in systems of the same pH, such as concentrated orange juice where the pH is always near 3.5. For the effect of changing pH see Rahn and Conn (1944).

Viable yeasts have been enumerated by plating on Difco Potato Dextrose Agar, cf. Ingram (1947c), and counting after three days' incubation at 25°C.(77°F.).

RESULTS

A. *Changes in Total SO_2*

1. *Initial Changes:* It is usual to find that if SO_2 is added to a juice, only a portion of it can be recovered. For this there are several causes. In the first place, the SO_2 may be added in the form of a salt which will usually yield only about 90 per cent of the expected quantity of SO_2 (some figures obtained were 93 and 90 per cent for $Na_2SO_3 \cdot 7H_2O$, 91 and 85 per cent for Na_2SO_3, and 93 per cent for $K_2S_2O_5$).

Next, the addition of such a salt to an acid juice results in a rapid evolution of gaseous SO_2, some of which is immediately lost to the atmosphere. If the juice is stirred to mix in the SO_2 this loss to the atmosphere is augmented, especially if warm, or when there is a small bulk of juice with a large surface, vigorously stirred (Table 1). Very similar losses have been reported during fruit preservation, Charley *et al.* (1942).

TABLE 1

Relation Between Duration and Intensity of Stirring and Loss of SO_2 From Concentrated Orange Juice Just After Addition of SO_2 in Various Forms[1]

Time of analysis after addition of SO_2	Slow stirring (60 r.p.m.) SO_2 added as			Rapid stirring (400 r.p.m.) SO_2 added as		
	$Na_2SO_3 \cdot 7H_2O$	$K_2S_2O_5$	SO_2 solution	$Na_2SO_3 \cdot 7H_2O$	$K_2S_2O_5$	SO_2 solution
0 hr.	350	317	330	331	307	315
1 hr.	339	299	328	322	317	332
2 hr.	333	297	325	335	301	269
4 hr.	300	270	318	245	250	196

[1] Figures represent p.p.m. total SO_2 determined by distillation. Experiments carried out with 250 ml. concentrated juice in 600-ml. beaker.

2. *Losses on Storage:* As is well known, after this rapid initial loss of total SO_2 the amount recoverable diminishes slowly as a fruit juice is stored. A similar change takes place in dried fruit, and it has been particularly well described for dried apricots, Sorber (1944), Stadtman, Barker, Mrak, and MacKinney (1946). The rate of loss from concentrated orange juice stored at various temperatures in open vessels is shown (Fig. 1); Table 2 gives figures for similar, but not exactly comparable, juices stored in screw-capped bottles.

Gaseous diffusion as an important possible cause of this loss is suggested by the fact that the rate of loss is much greater from open than from closed vessels (compare Fig. 1 with Table 2); and Table 1 shows that in favorable circumstances the loss by diffusion may be rapid. There

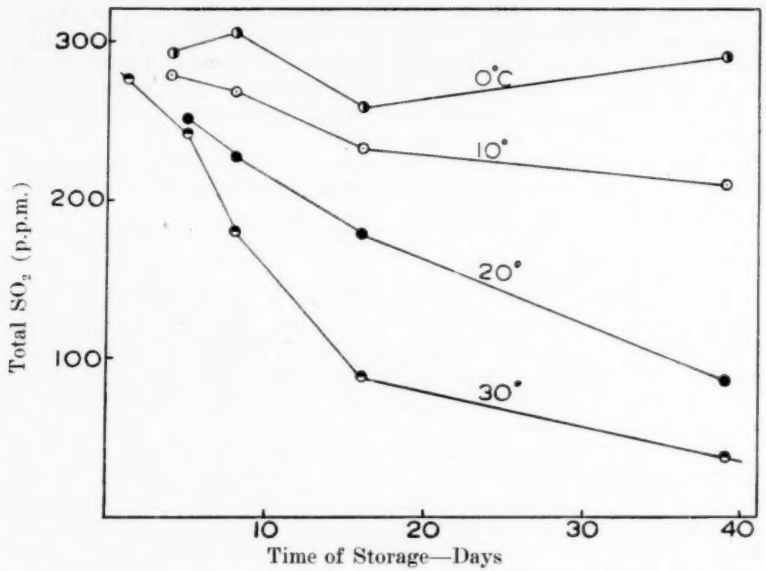

FIG. 1. Disappearance of total distillable SO_2 from concentrated orange juice stored in open conical flasks (750 ml. in 1,500-ml. flasks).

is, however, a substantial loss even under conditions where this cannot occur, as in the closed bottles.

Oxidation to sulphate by oxygen dissolved in the juice or included in the atmosphere of such bottles is a second possibility, but it seems hardly adequate to account for the observed losses. For example, in these bottles there are roughly 180 ml. concentrate, and a 10-ml. air-space. The air

TABLE 2

Total SO_2 in p.p.m. in Comparable Filled Closed Bottles (as in Text) of Sterile Concentrated Juice, Containing a Nominal 350 p.p.m. Initially, After Storage at Different Temperatures[1]

Sample I stored for 10 weeks at		Sample II stored for 3 months at		
0°C.	25°C.	20°C.	25°C.	30°C.
336	211	160	16(!)	51
328	222	138	240	112
310	221	342	320	16
320	218	64	269	80
304	222	224	169	167
320	209	240	167	.....
326	225	224	167	.....
327	224	244	.....	.....
317	227	218	.....	.....
349	205	.....	.....	.....
Average 324	218	206	195	85

[1] Samples I and II refer to two similar juices from different sources.

will contain 2.1 ml. O_2; the concentrate itself contains about 0.06 ml. O_2 (analyses kindly made by Dr. A. Pollard of Long Ashton Research Station gave 0.060, 0.015, 0.030, 0.025, av. = 0.033 ml. O_2 per 100 ml.). The total available O_2 is thus 2.16 ml., sufficient to oxidize 12.3 mg. SO_2, which could thus cause a loss from 180 ml. (236 g.) of concentrate of 52 p.p.m., which falls considerably short of some of the losses actually recorded. Moreover, in bottles of viscous concentrated orange juice left undisturbed with access of air, no consistent or significant difference has been found between the bottom layers and the surface layers, which should be more liable to loss by oxidation (Table 3). Finally, in fruit pulps the loss of SO_2 cannot be equated with the formation of sulphate (Table 4).

TABLE 3

Total SO_2 (p.p.m.) in Concentrate in the Top Half and Bottom Half of the Same Bottles of Concentrated Orange Juice[1]

Bottle	Top half	Bottom half
1	236	259
2	230	248
3	219	209
4	253	244
5	206	227
6	163	208
7	182	252
8	216	215
9	217	237
10	214	265
Average ± stand. dev.	214±24	236±21

[1] The bottles had been stored undisturbed for 10 weeks at 25°C. and contained a nominal 350 p.p.m. SO_2 initially.

It is necessary, therefore, to envisage mechanisms of loss other than those already suggested, and recently Sorber (1944) has shown plainly that such exist. He found that during 10 years' storage of dried apricots the total sulphur remained roughly constant at 2,300 p.p.m., the sulphate sulphur also at about 1,000 p.p.m.; while the distillable SO_2 fell from about 1,000 to 250 p.p.m. after three years and to about 100 p.p.m. after 10 years. Thus, after three years, of some 2,300 parts of sulphur only about half could be accounted for as sulphate and SO_2, the rest being in some non-volatile form not sulphate.

3. *Losses During Fermentation:* If a sulphited juice begins to ferment, it is common experience, cf. Downer (1943), that the effectiveness of the SO_2 in retarding this process seems suddenly to disappear. It is often supposed that this is because the SO_2 is carried away with the CO_2 liberated in the fermentation, compare Woodroof and Cecil (1945, p. 26); although this explanation is obviously inadequate, since the phenomenon may be observed in stoppered bottles where no CO_2 is lost to the atmosphere.

Direct experiment with orange juice has shown that the amount of SO_2 lost in this way during fermentation may in fact be only a small fraction of the whole, quite insufficient to account for the almost complete loss of its inhibitory power. The concentration of total SO_2 in the juice may decline hardly at all during the period of active fermentation (Fig.

TABLE 4

Comparison of Losses of SO_2, by Oxidation and Otherwise, From Bottled Fruit Pulps [1]

1	2	3	4	5	6
SO_2 added initially	Residual total SO_2	Total sulphate found	Less natural sulphate = sulphate by oxidation [2] of SO_2	SO_2 accounted for by residue + oxidation	Calculated loss other than by oxidation [3]
		(12 months' storage)			
Gooseberry (ca. 14°Br.)					
Na HSO_3 250	0	490	250(a)	250	0?
500	10	610	370	380	120
750	10	770	530	540	210
1000	70	810	570	640	360
SO_2 250	0	340	250(b)	250	0?
500	10	360	270	280	220
750	20	660	570	590	160
1000	120	580	490	610	390
		(12 months' storage)			
Apple (ca. 18°Br.)					
Na HSO_3 2000	Cold 70	400	150(c)	220	1780
2000	Htd. 440	430	180	620	1380
SO_2 2000	Cold 5	450	200	205	1795
2000	Htd. 140	440	190	330	1670
		(Stored 9 months, oiled paper covers)			
Strawberry (ca. 12°Br.)					
Na HSO_3 1500	930	270	20(d)	950	550
2000	1100	400	150	1250	750
SO_2 1500	550	410	160	710	790
2000	830	350	100	930	1070

[1] Quantities are given in terms of the equivalent SO_2, reckoned in p.p.m. [2] These data have been kindly supplied by, and published with the permission of, the British Food Manufacturing Industries Research Association. [3] It is assumed, for example at (a), that in pulp with 250 p.p.m. added SO_2 the whole of this was oxidized to sulphate: since sulphate equivalent to 490 p.p.m. was found there must have been sulphate equivalent to 240 p.p.m. in the pulp itself, which must likewise be deducted in column 4 from the quantities of sulphate formed with more than 250 p.p.m. SO_2 present. Similarly, at (b) the indicated deduction is 90 p.p.m.; at (c) and (d), in the absence of similar indication, we have assumed 250 p.p.m. of natural sulphate. It is, however, unlikely that added SO_2 would ever be wholly oxidized to sulphate as assumed, in which case more natural sulphate would have been present than has been allowed for in column 4: thus the figures in columns 4 and 5 may be too high, so that the residues indicated in column 6 should be minimal estimates.

2). Indirect evidence of the same thing will be shown (Fig. 7), where it may be seen that in comparable samples there was no significant difference in content of total SO_2 between bottles of juice which had just fermented and those which had not.

A noteworthy feature of the experiment (Fig. 2) was that the amount of gaseous SO_2 driven off by the CO_2 during fermentation appeared greatly to exceed the small diminution in total distillable SO_2 over the period of fermentation, or the relatively small loss of free SO_2 (70 to 45 p.p.m.) during the same period. It was, indeed, of the same order (75 p.p.m.) as the amount of distillable SO_2 which had disappeared in the period prior to the fermentation, which leads to the interesting specula-

tion that much of this extra SO_2 might have been produced by breakdown during fermentation of compounds (e.g., sulphates) formed by chemical transformation of the SO_2 during prior storage.

B. *Changes in Free SO_2*

1. *Approach to Equilibrium:* When sulphite is added to an acid juice, the SO_2 which remains in the juice is, of course, initially wholly in the free state; but it at once begins to combine with the sugars of the juice, and in a concentrated juice the major part is removed in this way, cf. Downer (1943).

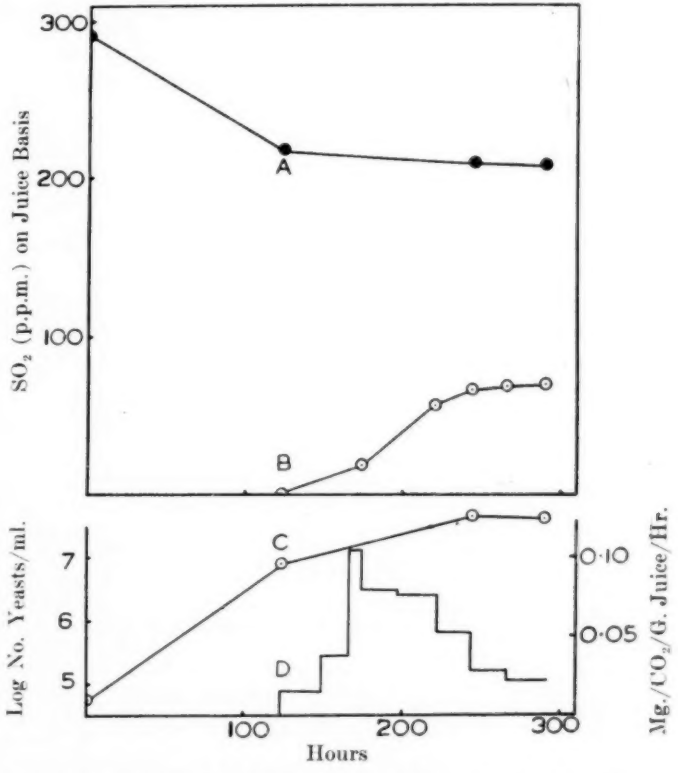

FIG. 2. Loss of SO_2 at 25°C. from orange juice as a result of fermentation.

A. Total distillable SO_2 (p.p.m.) remaining in juice.

B. SO_2 removed with CO_2 evolved (presumptive—estimated by passage through acid $K_2Cr_2O_7$ and back-titration).

C. Number of viable yeasts present.

D. Rate of evolution of CO_2 (estimated by absorption in baryta and back-titration).

NOTE: Although it is not certain that Curve B represents only SO_2, it does indicate the maximum possible loss.

Compared with subsequent changes the combination is quite rapid, being practically over in periods varying from a few days (Fig. 3), to a few hours (Fig. 6). The duration of this change is not greatly affected by temperature within the range studied (Fig. 3), but it does vary considerably from juice to juice, for reasons unknown—Figs. 3 and 6 represent roughly the extremes in this respect. On the other hand, temperature does markedly affect the final result: that the proportion of SO_2 remaining free is less, the higher the temperature, is shown in Fig. 3. These

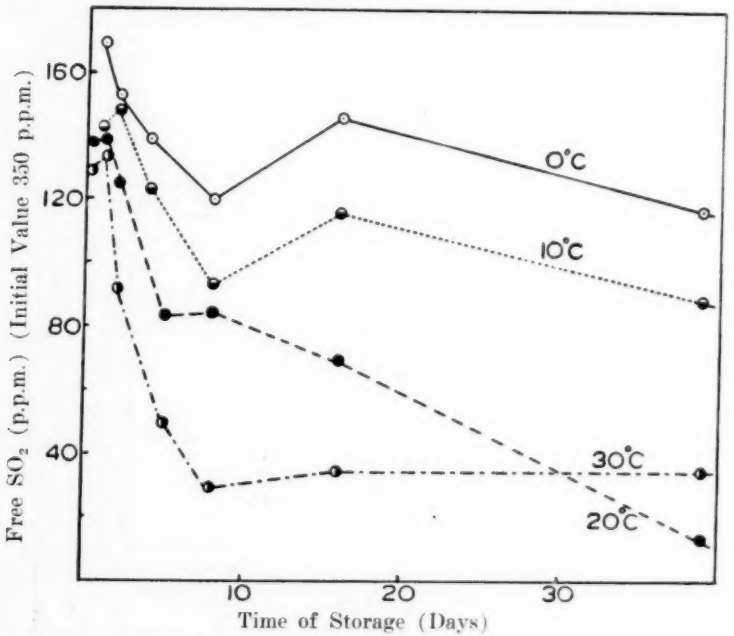

FIG. 3. Combination of concentrated orange juice with free SO_2 during storage of juice at various temperatures, after addition of Na_2SO_3 to give 350 p.p.m. total SO_2 initially.

NOTE: It is possible that the final value for 30°C. is spuriously high, for it is believed, Ingram (1947b), that heat treatment of a concentrate may cause overestimation of free SO_2 in it.

observations mean in practice that estimates of the amount of SO_2 which will remain free in a juice mean little unless one waits several days between adding the SO_2 and making the determinations, and the temperature must be controlled and specified. That at all ordinary temperatures a week should be sufficient is illustrated (Fig. 3).

It is not yet clear whether, under ideal conditions, there would be established after this initial change a true equilibrium, such as was demonstrated in the reaction between glucose and sulphur dioxide by Kerp (1907); but on present evidence it is reasonable to suppose that the much

slower changes which follow do represent the approach to an equilibrium, which is overtaken by other changes consequent on the storage of the juice, now to be described.

It should be noted that the ratio free/total SO_2 apparently rises with the quantity of total SO_2, compare Ingram (1947c), so that quoted values for this proportion would be of little value unless the amount of SO_2 present were also given, as in the data presented here.

2. *Changes on Storage:* As previously mentioned, there is a gradual loss of total SO_2; and this would lead to a corresponding diminution in the *quantity* of free SO_2 even if the proportion free did not alter.

There is in addition, however, a change which diminishes the proportion of free *to* total SO_2, which arises as a result of storage, and is similar whether the SO_2 is present throughout or is only added finally (Table 5). A moderate rise in temperature greatly accelerates the change, so that in concentrates stored for several weeks under warm conditions the amount of free SO_2 may be reduced to negligible proportions.

TABLE 5

Effect of Temperature on the SO_2-Combining-Power of Concentrated Orange Juice During Storage

Juice	Temp. of storage	Time of storage	Total SO_2 p.p.m.	Free SO_2 p.p.m.	Free SO_2 / Total SO_2
I. Sulphite added *after* storage.	°C.	*mo.*			
1	−1	4	350	85[1]	0.24
2	−1	4	350	85[1]	0.24
3	20	4	350	54[1]	0.15
4	25	4	350	44[1]	0.13
5	30	4	350	8[1]	0.02
II. Sulphite added *before* storage.					
1	0	1	320	130	0.41
2	0	1	320	138	0.43
3	0	1	307	96	0.31
4	0	1	304	100	0.33
5	25	2½	218	67	0.31
6	25	2½	224	63	0.28
7	25	2½	203	47	0.24
8	25	2½	212	41	0.19
9	37	6	85	<5	<0.06
10	37	6	79	<5	<0.06
11	37	6	83	<5	<0.06

[1] One week at 15°C. allowed for equilibration.

Under similar conditions (with no SO_2 present) the juice goes brown, and an inverse relation has been found between the browning of a juice due to heating and the proportion of subsequently added SO_2 which remains free (Fig. 4). It may be noted that, for a given degree of browning, the combining power of the juice was affected to a similar degree, whether the browning was caused by a short time at a high temperature or a longer time at lower temperature. This suggests that temperature alters the speed of the browning reactions, without substantially changing their nature.

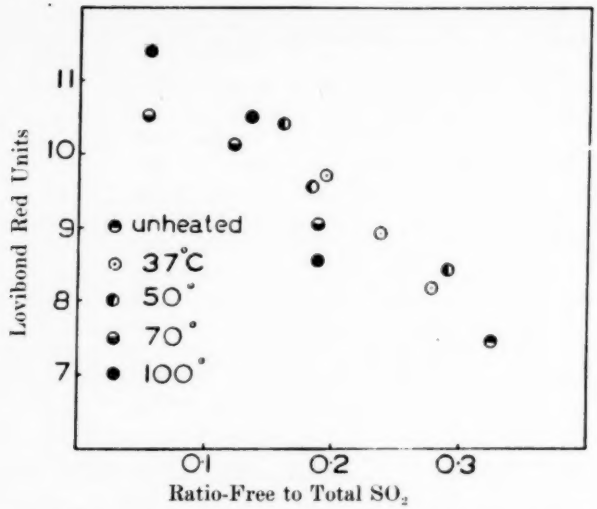

FIG. 4. Relation between the intensity of brown color developed on heating (at various temperatures) and the proportion which remained free of SO_2 added after heating. (350 p.p.m. SO_2 added and allowed to equilibrate at 15°C.) The measure of brown color used here is the number of red units needed to match the color of the concentrate (undiluted) in reflected light in a Lovibond Tintometer.

The same relation has been demonstrated with a wide range of juices, although obscured by inherent variations in color from juice to juice, in the form of an inverse correlation between brown color and the ratio free/total SO_2 (Fig. 5).

It seems probable that as a result of decomposition on storage, especially if heated, the components of the juice are broken down to give compounds such as aldehydes and ketones in greater quantity or with a greater affinity for SO_2 than the original sugars. These need not be the substances directly responsible for the brown color, but it seems that they may be produced by reactions associated with the process of caramelization; an example of such a substance is the hydroxymethyl furfural produced as an intermediate in the browning of dried apricots on storage or heating (personal communication from Dr. G. S. MacKinney).

Experiment has shown that the presence of SO_2 retards the development of brown color at high temperature, and it has a similar effect in dehydrated foods. It would be anticipated, therefore, if there is a relation between browning and loss of free SO_2, that after equivalent storage there should remain more free SO_2 if it is added initially than if added finally. The only data available (Table 5) indicate that this may well be so, but they are unfortunately inadequate to decide it definitely.

3. *Changes on Fermentation:* If yeasts are present and multiply so that the juice ferments, changes take place analogous to those caused by high temperature: the composition of the juice alters so as to diminish

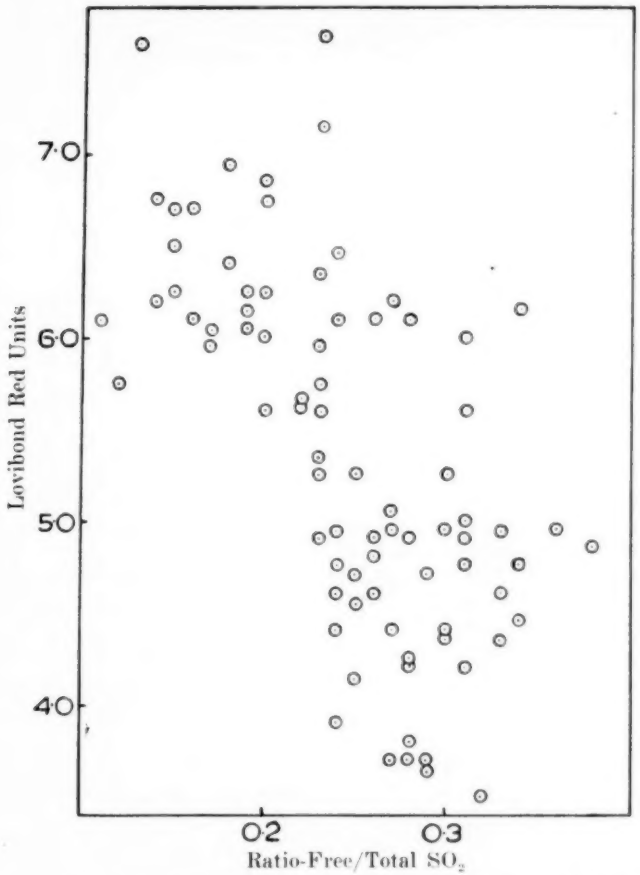

FIG. 5. Intensity of brown color in a comprehensive range of samples of U.S.A. concentrated orange juice, correlated with the proportion of added SO_2 remaining free. (350 p.p.m. SO_2 added and allowed to equilibrate at 20°C.—brown color as in Fig. 4.)

the proportion of free SO_2. The process responsible for this change, like that in browning, goes on whether SO_2 is present or not (Table 6).

It seems probable that this results from the production during fermentation of compounds which combine with free SO_2; examples might be acetaldehyde, glyceraldehyde, or pyruvic acid, all of which are intermediates in the fermentation metabolism. This explanation, originally suggested in principle by Müller-Thurgau and Osterwalder (1914), can be shown to be in harmony with several features of the behavior of the free SO_2:

(a) The SO_2 combines more completely in fermented juice (Fig. 6), (although the combination does not take place much more rapidly, nor

TABLE 6

Observations Showing That Fermentation Produces Similar Increase of SO_2 Combining-Power Whether SO_2 Is Added Before or After the Fermentation[1]

System	Yeasts/ml.	Total SO_2	Free SO_2	Free SO_2 / Total SO_2	Temp. of storage
Sound juice	0	250	70	0.28	25°
	0	336	151	0.45	0°
Fermenting juice 350 p.p.m. SO_2 added initially	4.7×10^3	225	65	0.29	25°
	6.0×10^5	242	68	0.28	25°
	6.0×10^6	259	27	0.10	25°
	2.7×10^7	258	19	0.07	25°
SO_2 added finally	7.0×10^5	280	9	0.03	25°
	1.0×10^6	250	35	0.14	0°

[1] Each group of observations refers to one of a number of bottles from one batch of concentrate, all stored for eight weeks.

is it complete, hence compounds such as acetaldehyde or acetone cannot be present in quantity).

(b) The free SO_2 does not begin to disappear rapidly until an active fermentation has begun, which is in these concentrates when the number of yeasts rises to about 10^6/ml. (Table 6). At this point, over a period when the total quantity of SO_2 changes relatively very little, the free SO_2 falls suddenly (Table 6 and Fig. 7).

(c) In the early stages of the development of such an infection, before the fermentation has begun, a moderate addition of a few hundred p.p.m.

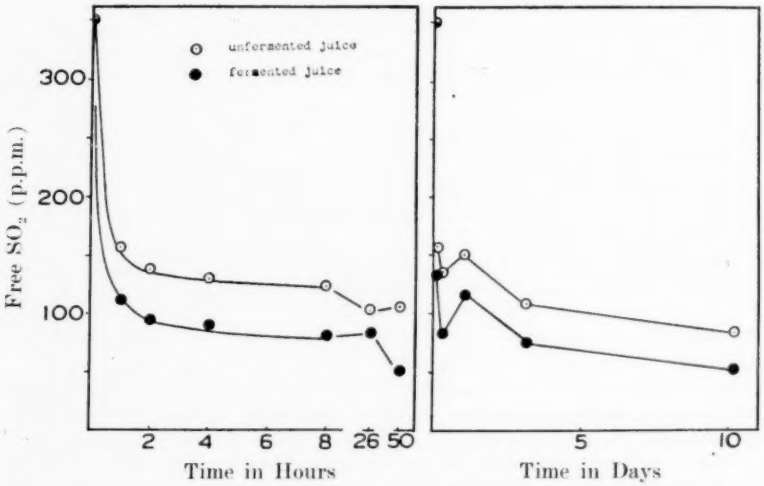

FIG. 6. More complete disappearance of free SO_2 from fermented juice than from unfermented juice (20°C.). Two different juices are represented here. The temporary over-combination, shown during the first few hours in the second juice, has been observed with some juices by other workers when trying out the technique of electrometric estimation with iodine. Its significance is not yet known.

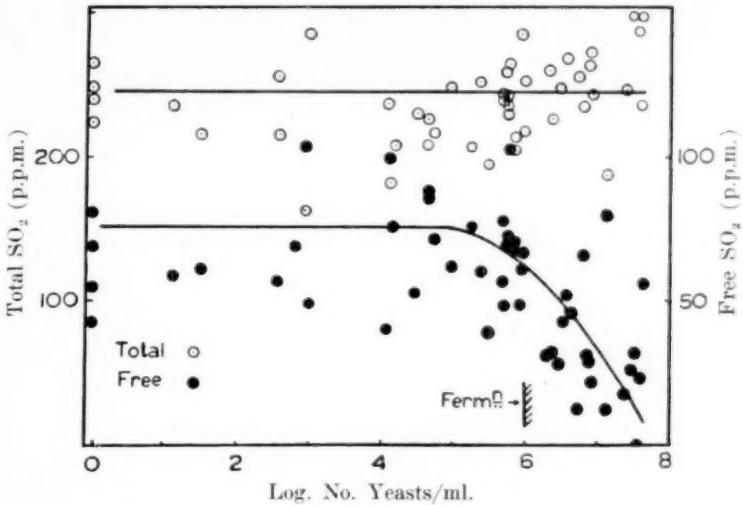

FIG. 7. Simultaneous estimation of total and free SO_2 in bottles of concentrated orange juice developing fermentation.

NOTE: The figure includes bottles stored at 25°C. for periods varying from four to 10 weeks, so that the normal changes consequent upon storage produced considerable scatter in the results. (Remember that the abcissa for total SO_2 on the left has only half the scale of that for free SO_2 on the right.)

of total SO_2 is usually sufficient to prevent any further development; but once fermentation has begun, very much larger additions of one or two thousand p.p.m. may be necessary (Fig. 8), and in the later stages of fermentation even this quantity may be ineffective. This is presumably because the added SO_2 combines with the products of fermentation, instead of attacking the yeast cells.

(d) The addition of fermented, to sulphited unfermented concentrate, renders the latter more liable to ferment, even if the fermented portion contains no living yeasts. This might well be because the fermented juice contains substances which remove the free SO_2 which is retarding the development of yeasts in the unfermented juice (Table 7, Experiment A).

(e) This may be coupled with the observations that pasteurization does not reduce the SO_2-combining-power of fermented concentrate to its level before fermentation (Table 7, Experiment A, and Table 8); and that the addition to sound juice of fermented juice with high combining-power will increase the combining-power of the former (Tables 7 and 9).

Most of these facts have been known for some time, but it is desirable that their implications in the practical handling of fermented juices should be recognized fully. Not only does pasteurization not restore the lower SO_2-combining-power which the concentrate had before it fermented but there is a danger that it may be pushed to the point of overheating the concentrate, resulting in a further increase in combining power be-

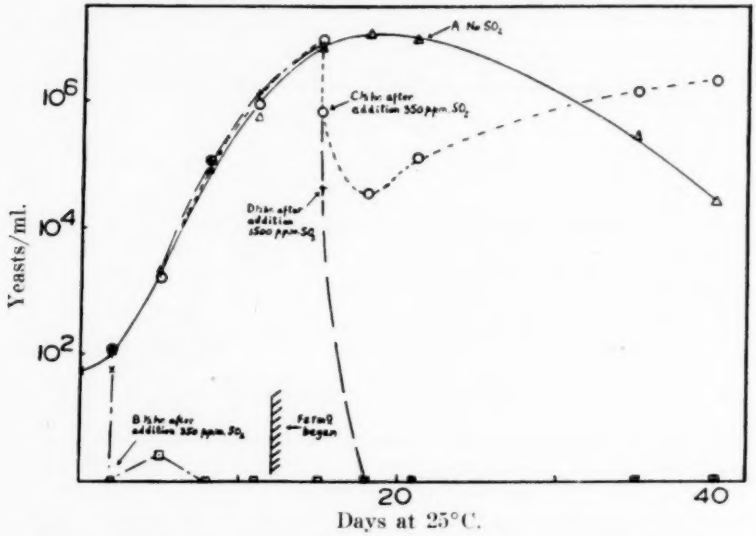

FIG. 8. The influence of fermentation in increasing the amount of SO_2 necessary to stop development of the yeasts.

A. No SO_2 added—normal course of fermentation.
B. 350 p.p.m. SO_2 kill all the yeasts within one-half hour.
C. 350 p.p.m. SO_2 retard but do not prevent development.
D. 1,500 p.p.m. SO_2 require two days to kill all the yeasts.

TABLE 7

Increased Tendency to Ferment (Indicated by Reduced Generation Time of Fermenting Yeasts), Caused by Addition of Fermented or Heated Concentrate to Fresh Concentrate

(Values in brackets inferred.)

Pasteurization treatment [1]	Free SO_2 (p.p.m.)				Generation time (days)				
					Mixture		Mixture		
	Fresh conctr. (1)	Fresh pastrzd. (2)	Fermd. conctr.	Fermd. pastrzd. (3)	(1, b) + (2)	(1, c) + (3)	(1, a)	(1, b) + (2)	(1, c) + (3)
(A) 1 min. at 90°C.									
11, Dec. 1946.....	63	67	53	50	....	....	.....	.....	.....
14, Jan. 1947.....	42	[45]	....	[25]	43	35	0.63	0.60	0.56
(B) Bottle to 90°C. (1 hr.).....	50	[<4]	....	[<4]	17	15	1.20	0.95	0.80

[1] Experiment A shows simply the effect of adding fermented material, for the flash-pasteurization did not affect the SO_2-combining-power. By contrast, Experiment B shows the result of inefficient pasteurization which further damaged the concentrate: the pasteurization was done by raising one-litre lots to 90°C. and cooling in water, the whole process, taking an hour. In either experiment homogenized sound concentrate was divided and (1) stored at 0°C., (2) pasteurized and stored at 0°C., (3) fermented and pasteurized; 350 p.p.m. SO_2 were then added to each and the free fraction measured after storing seven days at 25°C. (11, 12, 46). The first (sound) portion was then inoculated with a few fermenting yeasts, and when these had increased to 10^3/ml. (14, 1, 47) it was subdivided into three: a) had no treatment, b) was mixed with half its volume of pasteurized, fresh, and c) with half its volume of the pasteurized, fermented material. The free SO_2 and the number of yeasts in each mixture was estimated at once, and the trio of infected samples then returned to 25°C. in filled, closed bottles and the yeasts counted at brief intervals.

TABLE 8

Effect of Flash-Pasteurization of Unsulphited Concentrated Juice on SO_2-Combining-Power

(Figures are p.p.m. free SO_2 determined following storage for one week at 15°C. after adding 350 p.p.m. SO_2 at end of treatment.)

State of juice	Treatment		Free SO_2 (p.p.m.)		% fall
	Temp. (°C.)	Min.	Untreated	Treated	
Sound, nearly sterile	90-91	1¼ (lab.)	84	79	6
	87-88	2 (factory)	96	61	26
	83-84	2-4 (factory)	94	78	18
Begining ferment. 3×10^6 yeasts/ml.	86-87	1 (factory)	84	76[2]	10 ?
Fully ferment. 3×10^7 yeasts/ml.	90-91	1¼ (lab.)	25	50[1]	100 incr.

[1] The rise here may well have been due to loss of volatile aldehydes, etc., as the pasteurization was effected by flow through a system open to the air. [2] A similar thing might have happened partially in this case, too.

TABLE 9

Effect of Adding Fermented Concentrate on the SO_2-Combining-Power of Unfermented Concentrate [1]

Proportion of fermented : sound concentrate (pct.)	Free SO_2 attained in mixture			
	Example A		Example B	
	Calculated	Found	Calculated	Found
0	.....	116	.....	179
10	114	111	174	178
25	112	110	167	167
37	110	111	163	161
50	108	114	158	155
75	103	106	144	142
100	.....	99	.....	130

[1] Figures represent free SO_2 (p.p.m.) remaining after equilibration at 15°C., following addition of 350 p.p.m. total in Example A, and 450 p.p.m. in Example B. The free SO_2 diminishes in rough proportion to the quantity of fermented juice added.

yond that due to fermentation alone. In the rather extreme example represented (Experiment B, Table 7), pasteurization made the concentrates perceptibly more brown and the SO_2-combining-power was so much raised by the heating that little difference remained between the fermented and the unfermented concentrates; the addition of this material to fresh concentrate brought about marked acceleration of the onset of fermentation.

It will be evident that high-temperature storage, and fermentation, bring about very similar changes in the behavior of concentrates towards SO_2; and the production in both cases of aldehydic substances, with a relatively high affinity for it, would be sufficient to explain the corresponding increases in SO_2-combining-power.

DISCUSSION

These observations have an important bearing on the practices adopted in the commercial treatment of concentrated fruit juice.

There is ample evidence, both direct and indirect, that the inhibition of yeast development is due to some free SO_2 fraction rather than to that which is combined in organic bisulphite compounds. The direct evidence

lies in demonstration of correlation between germicidal power and free, but not combined or total SO_2, Ingram (1947c), and in the power of substances like acetone to remove free SO_2 and permit development of yeasts, Downer (1943). The indirect evidence has been summarized by Downer and linked with his observation that, when preserved with SO_2, concentrated juices (despite the relatively unfavorable osmotic conditions) seem to be more liable to ferment than corresponding dilute juices, which might be explained on the basis of the lower proportion of free SO_2 in concentrated juices.

The implication of these observations is plain: any treatment which reduces the proportion of free to total SO_2 will prejudice the protective action of sulphur dioxide which may be added as a preservative, at whatever time in the history of the juice. Data presented above enable one to point out treatments likely to do this.

In the first place, it is clear that fermentation of the juice at any stage will be most harmful (quite apart from changes in flavor, etc.). As has been said, it is well known that SO_2 is unlikely to preserve juice which is actively fermenting. It is now evident that this remains partly true even if the juice has been pasteurized after fermentation. Pasteurization may kill the yeasts responsible for the fermentation, but it does not remove the products of fermentation—indeed, on the contrary, it is now clear that if unskillfully done it produces more substances of a similar kind. Similarly, if the juice ferments before concentration, the harmful effects may partly persist after it has been concentrated. Finally, it has been shown that the addition of a small proportion of fermented juice, even if pasteurized, appreciably reduces the free SO_2 in a bulk of sound juice.

Processing should therefore be such that at no stage is there any opportunity for the development of microorganisms in any part of the juice, for their metabolic products remain in it, to combine with most of any SO_2 that may later be added. That this observation is relevant to current practice may be inferred from the facts, that in the production of orange juice it is not unusual to find infections of the order of 10^5 viable yeasts/ml. coupled with temperatures of 20 to 25°C.(68 to 77°F.) and storage tanks giving holding times measured in hours, while in most commercial orange concentrates direct microscopic examination reveals at once yeasts (usually killed by pasteurization, of course) in numbers at least of the order of 10^6/ml. In such circumstances it can hardly be doubted that the initial stage of fermentation will often proceed far enough in practice to appreciably influence the SO_2-combining-power of the juice produced.

A second important factor is the temperature treatment to which the juice is subjected. This is, of course, much more important with concentrated than with diluted juices, as the former are more readily damaged by heating. Concentration of the juice may involve exposure to temperatures reaching the order of 50°C.(122°F.) for several hours; and to ensure sterility it may be preceded or followed by pasteurization, involving exposure to still higher temperatures [usually about 90°C.(194°F.)] for periods up to a few minutes. Comparison of these treatments with the data reviewed (Table 10) will suggest that they are certain to have an appreciable deleterious influence on the SO_2-combining-power of the juice,

unless they are carefully controlled; and it is likely that the differences between the various juices (Fig. 5) were partly the expression of different time-temperature treatments adopted by different manufacturers. Even after preparation, storage of concentrated juice for a few days in warm conditions will noticeably increase its SO_2-combining-power. Obviously, therefore, if the juice is ever to be preserved with sulphite, it will be wise to restrict all temperature treatments to the minimum, since any excess will correspondingly reduce the level of free SO_2 which can be attained later.

TABLE 10

Summary of Effects of Various Time and Temperature Treatments of Concentrated (65° Brix) Orange Juice on Free SO_2 Remaining (at Equilibrium at 15° C.) After Addition of 350 p.p.m. Total SO_2 After Treatment[1]

Temp. (°C.)	Time of exposure to quoted temp.	Free SO_2 compared with that attainable in untreated juice (pct. less)
0	40 days	0
25	10 days	15
25	120 days	50
37	10 days	35
37	120 days	100
50	1½ days	10
50	3 days	45
50	6 days	50
70	6 hr.	40
70	12 hr.	60
70	18 hr.	85
83-84	2-4 min.	20
83-84	½ hr.	65
87-88	2 min.	25
90-91	1¼ min.	5
100	½ hr.	40
100	1 hr.	60
100	2 hr.	80

[1] These figures have been collected from divers experiments, made with similar but not identical juices, stored in various ways. They are not therefore exactly comparable one with another. It is also possible that where with severe treatments a large fall was recorded, the free SO_2 was overestimated and the fall was in truth still larger (cf. note to Fig. 3). The estimates are intended merely to indicate the order of magnitude of the change involved.

It is suggested that the relation between brown color and proportion of free SO_2 could be used to give an immediate rough guide to the quality of a juice in this respect, in the following manner: Evidence has already been given, Ingram (1947c), that if the free SO_2 in concentrated orange juice falls as low as 50 p.p.m. fermentation may develop even from very small infections such as can hardly be avoided in ordinary practice. If, therefore, such a concentrate is to be preserved by the addition of not more than 350 p.p.m. of SO_2 it is essential that the proportion of free SO_2 should not be less than 0.15 of the total. Comparison (Fig. 3) shows that, despite much variation in color from juice to juice, concentrated juices with less free SO_2 than this were so brown as to contain red color components equivalent to more than 6.0 Lovibond units. Thus, the use of only those juices less brown than this (i.e., scoring less than 6.0 units)

would insure the virtually complete exclusion of unsuitable material. (It is presumed that fermented juice would have been rejected in other ways.) It should be emphasized, perhaps, that these figures are intended merely as an example; they cover the variation in color encountered in juices of United States origin only, and with fruits from other sources with different color the figures might need modification—the same principle could, however, be applied, once the data had been accumulated.

Those treatments which are prejudicial to free SO_2 content are, of course, harmful in other respects; heating or fermentation cause loss of flavor and ascorbic acid, and brown color is in itself undesirable. As several of the essential characters: odor, flavor, ascorbic acid, and SO_2-combining-power, go largely hand in hand, there is some prospect of using any one of them as an index of the general quality of a concentrated juice. The development of this theme is reserved for a later communication.

In conclusion, attention may be drawn to the significance of the period of equilibration following the addition of SO_2. If a juice is liable to infection at some definite point in its processing, it will be best to add the preservative SO_2 as nearly at that time as possible. If this is done, most of the SO_2 (initially present entirely in the free state) will be available for a short time to attack the infecting microorganisms. If the SO_2 is added some time beforehand, only a proportion of it (possibly very little) will still be in the active form when infection occurs; while on the other hand, if the addition of the SO_2 is unduly delayed, the infection may have progressed so far as to cause chemical changes which will make most of the added SO_2 ineffective. Where infection may occur at several stages, the best plan would seem to be to handle at low temperature to minimize the activities of the microorganisms, and add the SO_2 as late as possible.

SUMMARY

Observations were made of the total (distillable) and free (iodine-titratable) sulphur dioxide in 65° Brix concentrated United States orange juices, under various conditions of treatment and storage.

As with other products, the recoverable amounts of total distillable sulphur dioxide diminished on storage. Losses were due in part to diffusion, and in part to oxidation; but as they still proceeded rapidly in closed vessels, it is suggested that a large part of the SO_2 was removed by some as yet unknown combination. The losses were accelerated by increase of temperature, especially above 20°C. During fermentation there was, contrary to general belief, no substantial change in the total SO_2.

With undamaged concentrate, from 65 to 75 per cent of the added SO_2 combined with the sugars, between 0 and 30°C.(32 and 86°F.), respectively. This change was relatively rapid, being practically completed in a few days. Thereafter, there was a slower fall in the free SO_2, due to two causes: first, the general loss of SO_2 noted above; and beyond this, storage changed the composition of the concentrate so that an ever-diminishing proportion of the total SO_2 remained free. This second change was greatly accelerated at temperatures above 20°C., and at the same time the concentrate went brown; there was a direct relation between the browning and the diminution in free SO_2.

Fermentation similarly changed the composition of the concentrate, so that of the total SO_2 a much smaller proportion remained free than before. It is believed that these changes following heating or fermentation arise from the production during such treatments of aldehyde-like substances.

It is pointed out that in the production of concentrated orange juice conditions are likely to arise in which such changes might proceed to an appreciable extent, which would make those juices more difficult to preserve with sulphur dioxide.

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DEVELOPMENT OF THIAMIN IN COOKING SWEET POTATOES

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Confusing results were obtained in studies to determine thiamin retention in certain processing procedures. Experiments designed to throw light on the data, indicated an actual increase in thiamin, as determined by the fermentometer, during the cooking of some vegetables.

It was considered that one possible explanation for the phenomenon in question is the degree of disintegration of the raw vegetable necessary to release cellular contents. Thiamin, being heat-labile, might well be expected to lead to confusion in such a study.

Since sugar can be determined accurately and is stable against oxidation and heat, it was chosen to follow the effect of degree of disintegration necessary to release cellular contents. It soon became evident that the degree of disintegration to release the maximum amount of sugar was not difficult to attain. The "Mikropulverizer," a hammer-mill type of machine, is equipped with a herringbone screen with $^{3}/_{64}$-inch slits and also with a screen having round holes of 0.02-inch diameter. Raw sweet potatoes cut in $^{3}/_{4}$-inch dice and passed with an equal weight of water through the Mikropulverizer, equipped with the latter screen, had their cellular structure broken down to a much more thorough degree than when equipped with the herringbone screen. This was plainly evident to the naked eye and also when the material was examined through the microscope. But the sugar content of the filtrate of the slurry was the same in both, and no more than in the filtrate of the slurry produced by the Waring blendor in two or three minutes.

However, if the diced sweet potatoes were first cooked and any evaporation made up with water, the sugar in the filtrate of the slurry produced in one minute in the Waring blendor was much greater than in the filtrate of the raw slurries. Moreover, if the raw slurries were heated to boiling temperatures the sugar content of the filtrate was then approximately that of the cooked, diced sweet potatoes. It thus became evident that cooking increased the sugar content of sweet potatoes, but it remained to throw light on the causative factor.

EXPERIMENTAL PROCEDURE

It is to be noted that after cooking it is difficult to obtain filtrate from sweet potatoes. Therefore, the slurry was diluted with an equal weight of 95 per cent alcohol, thoroughly mixed in the Waring blendor, and then filtered. To eliminate alcohol, a weighed amount of filtrate was then evaporated to a small volume, distilled water added, and again evaporated to a small volume, and then made up to half the weight of the alcoholic filtrate with distilled water, for sugar determination. While the raw

slurry did yield filtrate, it was likewise treated with alcohol and handled precisely as in the case of cooked sweet potatoes. This permitted an accurate comparison of the raw with the cooked material. In thiamin determinations the slurries could be used direct in the fermentometer and no filtration was necessary. The thiamin values are the average of duplicate determinations that were in satisfactory agreement. All sugar determinations were on filtrates as obtained above, after inversion, by the Munson-Walker method. That the copper reductant is actually sugar was demonstrated in fermentation tests. A different lot of sweet potatoes was used for each experiment. Results of typical experiments are given (Table 1).

TABLE 1

Raw vs. Cooked Sweet Potatoes

Raw sweet potatoes disintegrated	Thiamin content		Sugar content		Effect of cooking
	Raw	Cooked	Raw	Cooked	Pct. gain (g.) or loss (l.)
	Mg./100 gm.		Pct. in filtrate		
Expt. 1	.067	.076			13½ g.
In Waring blendor			.....[1]	.....[1]	....
Expt. 2	.052	.066			27 g.
In Mikropulverizer, using herring-bone screen			4.19	5.63	34 g.
Expt. 3	.069	.076			10 g.
In Mikropulverizer, using screen with 0.02-inch holes			3.35	4.62	38 g.
Expt. 4	.143	.108			25 l.
Slurry heated to boiling in 10 min. and boiled vigorously for 20 min.			3.71	4.85	31 g.
Expt. 5	.073	.093			27 g.
Slurry slowly heated to 96°C. in 10 min.			3.71	5.09	37 g.
Expt. 6	.121	.111			8 l.
Slurry slowly added to boiling mass during 10 min.			3.61	4.77	32 g.

[1] Not determined.

In Experiments 1, 2, and 3 (Table 1) the sweet potatoes for cooking were merely cut into chunks approximately one-inch thick and simmered for 30 minutes in an equal weight of water, then made into a slurry in the Waring blendor, while the raw were disintegrated (indicated Table 1) in each case with an equal weight of water. Evaporation during cooking was always made up with distilled water. In Experiments 4, 5, and 6, the entire sample of sweet potatoes, with an equal weight of water, was first made into a slurry in the raw state, with the Mikropulverizer, using the screen with 0.02-inch holes, and a portion of this was then taken for heating or cooking. In Experiment 6, to 200 c.c. of vigorously-boiling distilled water 1,500 grams of slurry were slowly added, the mass being kept boiling during the addition and well stirred to incorporate the addition quickly and to prevent losses due to spattering. Heating was done in

the lower piece of a quart-sized aluminum double boiler, the rounded sides of which prevented scorching on the sides and losses due to spattering. The 200 c.c. water used was evaporated during the operation, any excess evaporation being made up with distilled water. Some of this heated slurry was filled into No. 1 cans, sealed and autoclaved one hour at 115°C. (239°F.) but no further increase in sugar resulted, indicating the development of sugar is not a matter of simple heat hydrolysis.

To throw more light on the enzymic activity involved in sugar production in sweet potatoes, a mikropulverized slurry of raw potatoes, with an equal weight of water, was held at from 41 to 43°C.(106 to 109°F.) and periodically duplicate samples were withdrawn. In one of the duplicates, designated "R" (Table 2), the sugar in the filtrate was determined directly, while in the other, designated "C," it was determined after the slurry was dropped slowly into boiling water. It is evident there is only slight enzymic activity at the temperature employed, and the amount of sugar produced by boiling is not greatly affected by the time held previously at 41 to 43°C.

TABLE 2

	Minutes held at 41-43°C.			
	0	10	30	60
Per cent sugar in filtrate of R samples	2.26	2.40	Lost	2.62
Per cent sugar in filtrate of C samples	.....	3.04	3.20	3.08
Increase due to boiling (pct.)	.....	27.00	.....	18.00

In another experiment, kilogram amounts of the slurry were held at various temperatures for 45 minutes (see results, Table 3). The figures indicate an unusually high temperature for optimum enzymic activity and yet a lethal temperature materially below boiling. Part of the sugar increase at 90°C.(194°F.) may be ascribed to development at lower temperatures while the mass was being brought up to 90°C., for which five minutes were required.

TABLE 3

	Temperature (45 min.)				
	Original	45°C.	60°C.	75°C.	90°C.
Per cent sugar in filtrate	2.89	3.57	6.08	7.24	5.95

To throw light on the enzymic activity involved in thiamin production, a slurry of equal weights of sweet potatoes and water was divided into four portions which were held at varying temperatures, after which thiamin was determined. The 12-station fermentometer permitted running simultaneously the one- and two-microgram thiamin standards, each in duplicate, and the four portions of sweet potatoes, each with its sulfite blank. Four such experiments were made involving the Maryland golden, or big stem type, and the New Jersey, or little stem type, as well as old and new crop sweet potatoes. Results reveal no difference ascribable to these variants and are summarized (Table 4). The temperature at which each portion was held is in parenthesis.

It appears that maximum enzyme activity is at a much lower temperature for thiamin production than for sugar production, possibly as low as 40°C.(104°F.). There is some indication that continued heating, even at relatively low temperatures, destroys some thiamin. This, coupled with lack of a high degree of precision on the part of the fermentometer method, limits deductions from the figures.

TABLE 4

Expt. No.	Time held	Thiamin content *			
	min.				
1	30	.068 (room)	.088 (60°C.)	.098 (75°C.)	.098 (90°C.)
2	60	.066 (room)	.086 (40°C.)	.094 (60°C.)	.080 (75°C.)
3	60	.078 (room)	.102 (40°C.)	.074 (60°C.)	lost
4	60	.048 (room)	.096 (40°C.)	lost	.088 (75°C.)

* Mg. per 100 g. sweet potatoes.

DISCUSSION

Gore (1923) has presented data on the formation of sugar copiously when sweet potatoes are cooked in various ways. He was unable to completely prevent it when ground (in a Herles press), raw pulp was dropped into boiling water. Hesselbring and Hawkins (1915) demonstrated that a certain amount (independent of temperature) of starch is converted into sugar after harvesting, the "state of completion" being reached in 12 days at 30°C.(86°F.).

Balls (1947) summarized data of Magoon and Culpepper (1924) as follows:

CONTENT OF REDUCING SUGARS IN SWEET POTATOES

	Uncooked	Cooked
Freshly dug	2.26	8.19
	1.86	8.52
Stored 30 days	4.52	13.67
	4.14	11.11

Magoon and Culpepper offer no comments on the causative factor that converts starches to sugar, but Balls, without presenting further data, assumed it to be an enzymic reaction made possible by the slow heat penetration.

These various data present the peculiar phenomenon of sugar being developed (presumably from starch) at near boiling temperatures that are supposed to inactivate enzymes. Yet, continuing the heating at more elevated temperatures does not increase the amount of sugar. The fact that even more sugar is developed at lower than boiling temperatures, indicates that enzymes may in some way have been involved in preparing a portion of the starch so as to yield sugar at a near boiling temperature that permits no further enzymic activity.

Experiments presented indicate that thiamin, as determined by the fermentometer, is increased presumably by enzymic activity, when the temperature is brought to a favorable point. It is known, Nutrition Re-

views (1947), that the vitamins occur and function in complex combinations. Evidently, a portion of thiamin occurs in sweet potatoes in a form unavailable to yeast. Since heating also destroys thiamin, the quantitative effect of each factor is obscured. But since a 25 per cent increase was obtained by slowly bringing a sweet potato slurry to a temperature of 96°C.(205°F.) (Experiment 5, Table 1), while continued vigorous boiling (Experiment 4) resulted in a 30 per cent loss, it may be assumed there was some destruction during the 10-minute coming-up time (Experiment 5).

Some minor exploratory experiments indicate that these phenomena pertaining to sugar and thiamin are not restricted to sweet potatoes. It is hoped, in future work, to determine if this increase in thiamin, as determined by yeast fermentation, manifests itself in animal feeding experiments. Such a phenomenon may well be the basis for the superior performance of animals receiving canned foods, in comparison with those receiving similar raw foods, as noted by Kohman, Eddy, White, and Sanborn (1937).

SUMMARY

Data are presented to indicate that a portion of the sugar, developed when sweet potatoes are cooked, may be a direct heat effect, since sugar is formed when a pulverized slurry of raw sweet potatoes is slowly added to boiling water, a temperature that is generally expected to inactivate enzymes. This does not exclude the possibility that enzymes may have prepared a portion of the starch so that heat would convert it into sugar. Data from similar experiments suggest that thiamin, as determined by the fermentometer, is increased, presumably by enzymic activity, during the time for bringing sweet potatoes slowly to the boiling temperature.

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HISTOLOGICAL OBSERVATIONS ON THE SEED COATS OF SUCCULENT PEAS

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Nearly all histological investigations on pea seeds have been confined either to very early stages which involve embryo development, or to problems of seed-coat permeability and germination of ripe seeds, Hayward (1938). These stages are outside the significant range of maturity in the use of succulent peas for freezing. A few histological studies have been made on the succulent stages. Natural causes of toughening of skin tissues during maturation have been described, Reeve (1946a and b, and 1947). Pultz (1929) has discussed starch formation in the skins and cotyledons of a canning variety, the Alaska.

The present paper describes other changes that occur during maturation and includes the results of histochemical investigations. A brief survey has shown that the changes in structure and general composition described here are also characteristic of other common legumes. Because the purpose has been the gaining of fundamental histological information on the succulent stages of peas, no attempt has been made to study effects of environmental factors. Objective evaluations of quality, as affected by post-harvest conditions, have been reported by Boggs, Campbell, and Schwartze (1942 and 1943). A consideration of some of the factors of environment was presented previously, Reeve (1947).

MATERIALS AND METHODS

Fresh peas of several varieties named later were used. Some were obtained from a home garden, where it was possible to record age by tagging blossoms. Other samples were taken from commercially grown peas that had been iced for 24 hours following harvest. Sections from fresh peas, as well as fixed, paraffin-embedded samples, were used. Several different staining combinations provided equally satisfactory results, Reeve (1948). Histochemical methods are described later.

Sieve size is used here only as an approximation of maturity stages.[2] Sometimes peas of the same age and in the same pod fall into two or three different sieve sizes. In addition, growing conditions influence size.

Most of the observations were made on large-seeded varieties. However, except for size, medium- and small-seeded varieties develop in the same manner. In general, the young seeds of such large-seeded varieties as Laxton's Progress, Thomas Laxton, Giant Stride, and Alderman are not well filled until they reach Sieve Size 5. On the other hand, small-seeded

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[2] Commercial sieve sizes range from $^{9}/_{32}$-inch for Size 1 to $^{15}/_{32}$-inch for Size 7.

varieties, such as the Alaska, may be at "prime maturity" at Size 3, when the peas have fully formed cotyledons.

GENERAL CHARACTERISTICS OF MATURING PEAS

The embryo occupies 10 to 50 per cent of the total volume inside the seed coat at Sizes 1 to 3 in large-seeded varieties. By the time Size 1 is reached most of the embryo is composed of the cotyledons. The remainder of the space is filled with a watery fluid characteristic of immature peas. This fluid consists partly of a nutritive endosperm and contains an "enzyme complex." In large-seeded varieties this fluid may be present even in peas of Size 4. It has been generally assumed that the endosperm contributes to the sweetness and tenderness of peas until they are more than half grown, Hayward (1938). This assumption oversimplifies a complex of interrelated factors. For instance, the embryos of immature peas are apt to be proportionately high in sugars and low in starch when compared with embryos of mature peas.

Pultz (1929) states that the endosperm in Alaska peas is entirely absorbed by the embryo by the twelfth day following fertilization. However, as with size, age in days is only an approximation of degree of maturation. Some varieties develop more rapidly than others, and environment also influences the rate of maturation.

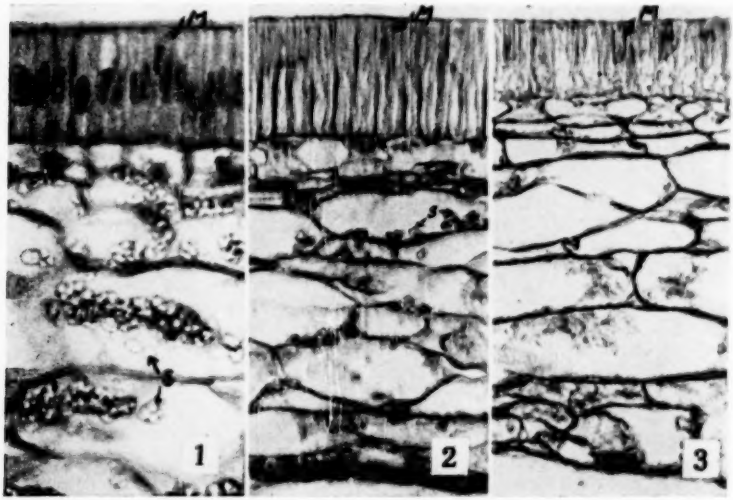
HISTOLOGICAL CHARACTERISTICS AND RESORPTION OF SKIN TISSUES DURING MATURATION

The skin of the pea seed develops from the outer integument of the ovule and is composed of several different tissues, Hayward (1938). The two outermost layers become highly specialized in wall structures which influence texture, Reeve (1946a and 1947). Most of the remaining tissues are composed of parenchyma cells (Figs. 1 to 3). The innermost cells of this tissue are resorbed during maturation of the seed. At approximately Sieve Size 5 in large-seeded varieties, resorption has nearly ceased (Figs. 4 to 6). The ripe seed has a skin composed mainly of the two outer layers and a thin underlying layer of parenchyma through which small strands of conducting tissues have developed.

In mature peas the inner surface of the skin is always bounded by a residuum of partly digested cells and other products of the enzymatic processes of the endospermous fluid. This residuum is very thin and could escape observation in sections prepared by methods which do not differentiate it well. In the histological sense, full development of the skin tissues is not reached until the peas are nearly ripe and approaching dryness. Before this stage, however, the cotyledons become hard and starchy, and the embryo is virtually dormant. The major changes in structure and composition of the skin occur during growth in the more succulent stages.

Although resorption of the inner integument has been recognized previously, and is assumed to occur in certain other legumes, Hayward (1938) and others, this subject has received little histological attention. It has not been generally known that the process of resorption in peas involves maturity stages used in food processing.

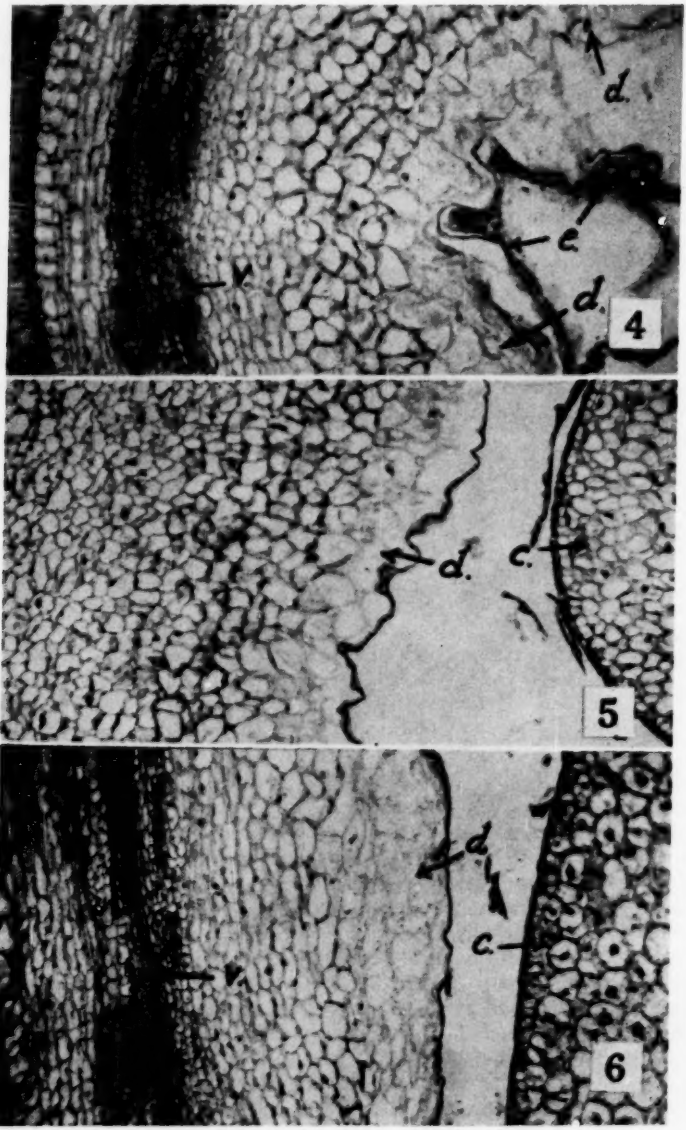
Generally, it has been stated that the inner integument is resorbed during early growth of the ovule. No published studies have described the same process as occurring in the tissues of the outer integument. Hayward (1938) and Pultz (1929) both show that the inner integument is completely resorbed on or before the fifth day after fertilization. This is well before the seed has reached Size 1. Because most varieties do not reach harvestable age until 10 to 14 days after flowering, it is evident that continued resorption, during most of seed growth, occurs in the parenchyma of the outer integument. In the varieties investigated, no



FIGS. 1-3. Photomicrographs of skin tissues at different ages (sections cut from the lateral sides of the seed, i.e., next to the walls of the pod); M—macrosclereids (epidermal cells); S—starch grains in parenchyma cells; note differences in magnification. Fig. 1—From pea slightly younger than Sieve Size 1, 400 ×. Fig. 2—From pea about Size 4, 200 ×. Fig. 3—From mature or nearly ripe seed, 200 ×.

evidence of an inner integument could be found in stages more than four or five days beyond flowering. At this stage the cotyledons are not evident and the embryo is a small sphere of actively dividing, growing cells. Subsequent development of the embryo is rapid and probably is influenced by the rate at which cells of the outer integument are digested and resorbed.

During the processes of tissue digestion and resorption along the inner surfaces of the skin, other changes occur. Starch grains are formed in the parenchyma cells of the skin during early stages (up to Size 2) more rapidly than in the cotyledons. A similar condition was described by Pultz (1929) for Alaska variety. During resorption, starch grains, other cell contents, and cellulose walls of the innermost parenchyma cells are digested; the soluble carbohydrates are then available either to the growing embryo or the outer skin layers (sclereids), where specialized cell



FIGS. 4-6. Photomicrographs of sections through skin tissues showing partly digested tissue "d" along the inner surface of the skin; C—cotyledon; E—endosperm; V—vascular or conducting tissue through which nutrients are translocated from the parent plant. Magnification 100 X. Fig. 4—From pea about Size 1. Fig. 5—From pea about Size 2 or 3. Fig. 6—From pea about Size 4.

walls also are being developed. Starch formation in the skin rapidly diminishes beyond Size 2. The skin tissues contain little visible starch by the time the pea is beyond "prime maturity" (compare Figs. 1 to 3).

Comparison of successive stages of maturity as to total skin thickness, at a given region, and size and number of undigested cell layers, shows that rate of tissue resorption is rapid. Examination of serial sections from peas of successive ages revealed a marked decrease in average number of undigested cells. Actually, there was not a corresponding decrease in total skin thickness. In very young peas, and often up to Sizes 2 and 3, some parenchyma cells divide and add new tissue, while others are being digested. Cell division is pronounced in stages preceding those represented by Sizes 1 and 2. At Size 2 or 3 only occasional cell divisions could be found.

As previously stated, cell enlargement also occurs in these stages and continues until the seed is nearly ripe. At Size 6 or 7, the total thickness of the skin at the lateral sides of the seed (next to the pod) is only six to eight parenchyma cells and the two outermost layers (sclereids). The total thickness at this age, however, may be only slightly less than for the skins of younger peas because of the degree of cell enlargement that has occurred (Figs. 1 to 3). It was previously shown, Reeve (1946a) that from Size 1 to 7 the outer surface area of the skin increases three-fold. Beyond Size 2 virtually all growth in skin tissues is by cell enlargement, and during the same time the sclereids are developing their characteristic, thickened cell walls.

In consideration of the specialized tissue formation, the close correlation between the growth of these tissues, the embryo, and the processes of tissue resorption, it is evident that the skins of peas present quite special, inherent conditions. Since these histological processes involve embryo growth as well as development of skin tissues, they influence the natural qualities of whole succulent peas.

DEVELOPMENT OF HISTOCHEMICAL METHODS

Histochemical tests previously made to characterize the cell walls of the sclereids, Reeve (1946a), led to an extended study of other factors of composition associated with maturation. Aldehydic materials were found to be abundant in the skins. Because reagents commonly used to detect aldehydes also react with enzymes (particularly peroxidase), it is necessary to consider the conditions of these tests in detail.

It has been generally recognized that the peroxidase testing reagents, benzidine and guaiacol, in the presence of hydrogen peroxide, give strong reactions in the seed coats of peas even after they have been subjected to prolonged scalding. With benzidine, color reactions other than the true peroxidase color ("benzidine blue") result. Such reactions may be found in other vegetables. The colors, yellow to red-brown, are readily seen in tissues containing lignified cell walls, such as xylem, or in other regions in which the cell walls are cutinized or suberized. In these tissues, aldehydic compounds are associated with the formation of cell wall materials. In addition, oxidative processes during cell growth and elaboration

of cellular products may produce transitory aldehydes. These generally are soluble, and color reactions with them are diffuse.

Van Fleet (1942, 1943, 1945, and 1946), in investigations of oxidation systems in the endodermis of roots, used benzidine and guaiacol, and other reagents under conditions that demonstrated the presence of aldehydes in specialized endodermal cell walls. Crocker (1921) has described aldehydic reactions of various reagents used for detection of lignin. Under certain conditions, rosaniline dyes, when decolorized with an excess of sulfurous acid, may be used for the cytological detection of aldehydes, Bourne (1942).

With guaiacol, both peroxidase and aldehydic materials give almost identical color formations. Consequently, misinterpretation of peroxidase tests might result when this reagent is applied directly to vegetable tissues. It therefore seemed desirable to conduct a series of histochemical tests on color reactions obtainable with peroxidase testing reagents. For this purpose paraphenylenediamine reagents were adapted from the spot test for aldehydes described by Feigl (1939). Paraphenylenediamine is oxidized by peroxide to form a black compound, Bandrowski's base. Aldehydes increase the velocity of the reaction and, according to Feigl, the reagent may be used under certain conditions to detect and differentiate aliphatic and aromatic aldehydes.

The reagents described by Feigl consist of two *N* acetic acid, two per cent paraphenylenediamine, and three per cent hydrogen peroxide. At time of application, these are added in equal parts to the test solution. Because of the formation of innumerable bubbles when three per cent hydrogen peroxide is applied to plant tissues, the test was modified to yield results that did not obscure structural details. It was found that 0.05 per cent or less hydrogen peroxide was suitable. Also, solutions of 0.5 to two per cent paraphenylenediamine gave nearly identical results. When these modifications were verified with known aldehydes, no significant differences in reactions could be detected. Instantaneous color reactions were obtained except in tests made with extremely diluted aldehydes and with less than 0.05 per cent hydrogen peroxide. In these extreme dilutions, color formation occurred within a few seconds.

In addition, both the original and the modified reagents were used with and without the acid for testing a peroxidase preparation known to be active. Equal volumes of the reagents and diluted peroxidase solution were mixed. Strong, positive, black reactions were obtained when the acid was omitted. In the presence of acid, however, only a gradual browning developed after several minutes. This reaction eventually terminated in a black compound. It does not seem likely that an active peroxidase text would be obtained at this acidity. However, some reaction with a porphyrin residue might be expected. These results are compared with those obtained with other reagents (Table 1).

Further tests were made with heated and KCN-treated peroxidase solutions (Table 1). The heat treatment was immersion in boiling water for one, two, and three minutes. Very slow reactions were obtained with some of these, and those samples heated for five minutes gave very slow reactions with paraphenylenediamine, which were comparable to discoloration

in the control. The KCN treatment consisted of adding about three drops of five per cent KCN to about 20 ml. of peroxidase solution. Both acid and neutral solutions of the reagents were used for testing these samples after 10 minutes. In these a slow darkening, comparable to that of the controls, occurred over a period of 30 to 40 minutes. It is possible that the slow color development in the heated samples was a reaction with a porphyrin residue when color development exceeded that of the controls.

HISTOCHEMICAL INSPECTION OF THE SEED COATS

Best results for microscopic examination were obtained with sections 10 to 20 microns thick cut on a freezing (CO_2) microtome. However, tapering hand-cut sections were satisfactory for more general observations after a critical study of reactions in cellular structure had been made. Naturally, in the thinner sections the depth of color was much less than in thicker sections; also, the thicker sections presented more of the diffuse color formations which tended to mask those reactions associated with structures.

TABLE 1

Reactions With Potato Peroxidase

Reagents + H_2O_2	Unheated	Peroxidase treatment—heated			KCN
		1 min.	2 min.	5 min.	
Guaiacol	+	—[1]	—	—	—
Guaiacol + 2N acetic acid	—[2]	—	—	—	—
Benzidine	+	—[1]	—[1]	—	—
Benzidine + 2N acetic acid	—[2]	—	—	—	—
2% paraphenylenediamine	+	—[2]	—[1]	—[1]	—
2% paraphenylenediamine + 2N acetic acid	—[2]	—[1]	—[1]	—[1]	—
0.5% paraphenylenediamine	+	—[1]	—[1]	—[1]	—
0.5% paraphenylenediamine + 2N acetic acid	—[1]	—[1]	—[1]	—[1]	—

[1] Faint color formation after standing 10 to 15 minutes (possibly a reaction with porphyrin residue but generally comparable to discoloration of control). [2] Slight color formation after standing 10 to 15 minutes, slightly darker than control, possibly a porphyrin reaction.

Whole peas, or the separated skins, were steamed one, two, and three minutes, cooled, and sectioned immediately. In addition, sections of raw fresh peas were gently heated on a microslide over a microburner for (a) about 30 seconds or (b) until steam arose or minute bubbles formed in the water (about 90 seconds). Since heat transfer in 10 to 20 micron sections can be considered instantaneous, greater uniformity of enzyme inactivation would be obtained by this than by the former treatment. "Poisoned" sections were prepared by soaking raw sections in either one or five per cent KCN for several minutes. These sections were then washed in distilled water before testing, although a few were tested in the presence of KCN and it was found that the aldehydic reactions appeared to be little affected by the KCN.

Sections of peas from different varieties showed no differences when tested with the various reagents. However, raw, heated, and KCN-treated sections gave distinctive colors by which it was possible to distinguish sharply between the aldehydic and peroxidase reactions (Tables 2 and 3). Although the modified tests with paraphenylenediamine provided the best

TABLE 2

General Initial Color Reactions in Skin Tissues[1]

Reagents + H_2O_2	Raw sections	Heated sections		KCN sections
		30 sec.	90 sec.	
Benzidine	+ p (bluish) + a (red-brown)	− p, + a (red-brown)	− p, + a (red-brown)	− p, + a (red-brown)
Benzidine + 2N acetic acid	− p, + a	− p, + a	− p, + a	− p, + a
2% paraphenylenediamine	+ p, + a (black, green, and red-brown)	− p, + a (green and red-brown)	− p, + a (green and red-brown)	− p, + a (green and red-brown)
2% paraphenylenediamine + 2N acetic acid	− p, + a (green, red-brown and yellow)	− p, + a (green, red-brown and yellow)	− p, + a (green, red-brown and yellow)	− p, + a (green, red-brown and yellow)
0.5% paraphenylenediamine	+ p, + a	− p, + a	− p, + a	− p, + a
0.5% paraphenylenediamine + 2N acetic acid	− p, + a	− p, + a	− p, + a	− p, + a

[1] + p = positive peroxidase, + a = positive aldehyde. Note: Guaiacol, which gives a bluish oxidase reaction, shows no color differentiation between aldehydic and peroxidase reactions, both being red-brown in the presence of H_2O_2.

TABLE 3

Nature of Aldehyde Reactions in situ

Reagents	Raw sections	Heated sections	KCN sections
Benzidine	+ a masked rapidly by + p; all colors black in 10 to 15 minutes	+ a instantaneous; slight + p after 10 or 15 minutes in 30 sec. sections	+ a instantaneous
Benzidine + 2N acetic acid	+ a instantaneous, slight + p after 5 or 10 minutes	+ a instantaneous	+ a instantaneous
Paraphenylenediamine	+ a masked by + p; all reactions black in 2 to 5 minutes	+ a instantaneous; all reactions black in 5 to 10 minutes	+ a instantaneous
Paraphenylenediamine + 2N acetic acid	+ a instantaneous; no residual porphyrin reaction evident, or if later present, masked by + a. Black in 10 minutes	+ a instantaneous, becoming black in 10 to 15 minutes	+ a instantaneous becoming black in 10 to 15 minutes

determinations of localized reactions, no differences in over-all color reactions could be discerned between results with the original and the modified tests. Likewise, no differences could be found between the one and the five per cent KCN-treated sections. In all the heated and "poisoned" sections, distinctive localized color reactions were obtained with both the acid and neutral solutions of the testing reagents. These color formations were characteristic of those described by Feigl for aldehydes.

Differences between aldehydic and peroxidase reactions can be better understood when related to certain histological details. All the positive peroxidase reactions were diffuse; that is, instantaneous color formation occurred in cell solutions and in the medium surrounding the tissues. Later color development, or further darkening, became associated with structural components by the absorption of some of the precipitated compounds on cell wall surfaces, etc. In tests which were considered negative for peroxidase, a similar color formation occurred in the heated sections only upon standing 10 to 15 minutes.

Because some of the aldehydic reactions in the heated sections darkened within several minutes, it was not possible to distinguish a porphyrin reaction with any degree of certainty unless such sections were carefully compared with the results in KCN-treated sections. It appears likely that a porphyrin reaction did occur in all heated sections after standing several minutes, since these later color changes were more pronounced in heated than in KCN-treated sections. In addition, all chloroplasts showed some color formation in the heated sections, as might be expected with porphyrin-related reactants in the chlorophyll.

Aldehydic reactions sharply contrasted with those of peroxidase, both in color when the paraphenylenediamine reagent was used, and in association with cell structures. Raw sections tested in the presence of two *N* acetic acid did not develop a blackening reaction for five or more minutes, during which time aldehydic color formations were pronounced. Outer cell layers and the sclereid cell walls instantly became an intense green. The green slowly changed to red-brown and then black. The black developed more slowly in heated and in "poisoned" than in raw sections when the tests were made without the acid. There was little or no difference in the rate of blackening in sections tested in acid solution. Thus, although the black reactions of peroxidase and aldehydes are more or less the same in appearance, differences in rate can be detected by comparing results in acid and in neutral solutions.

In the parenchyma tissues, instantaneous reactions with paraphenylenediamine ranged from intense green near the outer layers to yellow-brown or orange in the inner regions. The lighter yellow was scarcely evident in sections treated with neutral solutions, but was clearly evident (although quite transitory) in sections treated in acid solution. These yellows changed slowly to turbid brown and gray-black in several minutes, during which time the intense green also changed to black. Part of the red-brown and apparently all of the yellowish reactions were diffuse. The change to black in the KCN-treated sections was slow by comparison with raw and heated samples. Because KCN inhibits reactions with porphyrin, it seems likely that all the color reactions in the poisoned sections were aldehydic.

Initial aldehydic reactions with benzidine were not so striking. However, the red-brown contrasted with the "benzidine blue" of the positive peroxidase. Comparisons of results with benzidine and those with paraphenylenediamine showed no "benzidine blue" in sections treated with KCN and also in those heated sections in which aldehydic reactions with the paraphenylenediamine were obviously dominant.

Comparisons of mature and immature peas showed only slight differences *in situ* when sections were tested with paraphenylenediamine. Slight localized differences were observed, in that the older peas with more pronounced sclereid walls naturally showed a more pronounced aldehydic color formation associated with the specialized cell walls. In very young peas with no specialized cell walls, only a slight green reaction was observed. However, the yellowish reaction in the zone of digested tissues was more pronounced in young peas in which tissue resorption obviously was more active. In older peas in which tissue resorption had virtually ceased, the yellow coloration was faint or absent. An instantaneous greenish-brown reaction was observed in the previously described residuum.

An attempt was made to study the aldehydic reactions with respect to influence of delay after harvest. Several lots of fresh, unshelled peas at different ages were held at room temperature for periods of four hours to several days for comparisons with freshly harvested samples. However, the results were inconclusive. Filtrates prepared from the skins of the same lots showed a tendency for aldehydic reactions to be more pronounced in samples subjected to longer delay periods. These reactions would correspond to the diffuse reactions in the sections. It appears that if changes do occur in the aldehydic materials during delay, the total of reactant materials is not appreciably affected because many of them are associated with cell wall substances.

For comparisons with the skins, raw and treated sections of cotyledons were tested with the benzidine and paraphenylenediamine reagents. In neutral solutions the raw sections showed positive, somewhat localized, peroxidase reactions which were much less intense than in skin sections. The most pronounced "benzidine blue" and black (with paraphenylenediamine) occurred in procambium (embryonic conducting) tissues of the embryo. In addition, the growing points (apical meristems) of epicotyl and hypocotyl showed more intense reactions than did the storage parenchyma of the cotyledons. Aldehydic reactions in the heated and KCN-treated sections were much less intense than in the skins. Instantaneous green developed along the epidermis of the cotyledons, particularly in sections of older peas where the epidermal cells showed a definite cuticle. Lighter brownish reactions occurred in the meristematic tissue zones (growing points and procambium). All nuclei darkened appreciably, probably because of reactive material in the nucleic acid. None of the reactions was comparable in intensity to those obtained in the skins.

Although the paraphenylenediamine reagent tends to discolor after short exposure to air, such discoloration is not detectable under the microscope. However, fresh reagent was prepared for each series of tests to insure uniformity of treatment. Results obtained with paraphenylenediamine indicate that both the original and the modified tests, as used

histologically, specifically differentiate between peroxidase and aldehydes. Feigl (1939) considers the yellow reaction, in the presence of acid, to be characteristic for aromatic aldehydes.

DISCUSSION

Although it was not possible to demonstrate a direct relationship between aldehydes and off-flavor in delayed peas, such histological conditions as tissue resorption are significant. This significance is substantiated by the results of some brief food appraisal tests conducted at this Laboratory. When the skins and cotyledons of fresh and delayed frozen peas were separated, puréed, and subjected to a taste panel, it was obvious that off-flavor in the delayed samples predominated in the skins. Although aldehydes might be a contributing factor, these investigations show they are also abundant in fresh peas.

Maturation involves a series of interrelated histological changes. Certain of these are more pronounced than others at different stages. For instance, tissue resorption in the skin continues until the seeds are well filled, but is most pronounced in immature peas. At Sieve Size 4 (in large-seeded varieties) cell-wall thickening in the sclereids is well established. At Size 5 this wall thickening has become pronounced and influences texture, but at this stage the rate of tissue resorption has sharply diminished. Peas at "prime maturity" are approaching conditions necessary to ripening of the seed; the cotyledons are fully formed and are full of starch, but their tissues have not yet hardened. Except for increased toughening of the skins, such peas are still succulent. However, "prime maturity" is confined to a relatively narrow range of histological development.

It is commonly recognized that a given variety possesses characteristics of flavor and texture that vary according to age of fresh peas. Although histological factors of structure and composition may be correlated with these natural qualities, there is no obvious and simple relationship to quality in the processed commodity. Nielsen and co-workers (1947) have suggested that certain flavor characteristics may be associated with immaturity. It is possible that structural changes which occur during maturation could affect the processed product under certain conditions of post-harvest handling. For instance, when peas are shelled, the normal supply of nutrients to the seeds is severed; the processes of growth in the seed tissues, however, continue under altered conditions. Certain changes involved in tissue resorption in the skins may become accentuated during delay after harvest. Since tissue digestion involves not only cell wall carbohydrates but also entire cell contents, which include proteins and fats, it is obvious that this process must influence certain quality characteristics.

Because texture is an important factor in freezing, Nielsen and co-workers (1947) have also suggested (1) the desirability of selecting varieties with more tender skins at maturity and (2) the use of tenderizing methods. Although certain chemical treatments provide a tenderizing effect by their action on the intercellular substances of the middle lamellae, there are significant histological limitations, Reeve (1946a and 1947). There also may be limitations to the practicality of varietal selections based only on

texture characteristics. Changes reported here indicate that peas with more tender skins at maturity might also retain some of the undesirable characteristics of immaturity. This condition might result if, at a suitable maturity for processing at a succulent stage, peas possessing sclereid cell walls not fully developed also retain an active phase of tissue resorption along the inner surfaces of the skin tissues. Thus, varietal selections should be based upon interrelated factors.

SUMMARY

A study of histological development in peas of succulent stages has shown that tissue digestion occurs along the inner surface of the testa or skin. This process begins in the very young ovule and the inner integument is completely resorbed while the embryo is small. During succulent stages, tissue resorption continues along the inner surface of the outer integument at an appreciable rate until the peas are mature.

Starch grains are abundant in the skin until the seed is nearly full size. Few or no starch grains were observed in the skins of mature peas.

Histochemical tests were developed to demonstrate the presence and distribution of aldehydic materials in peas. The same reagents also could be used to demonstrate peroxidase activity. With inhibitors (such as KCN), heat treatment, and acid, differential reactions which distinguished between peroxidase and aldehydic materials were obtained.

With paraphenylenediamine, both in acid and in "neutral" solution, distinctive color reactions were pronounced in the skins and faint in the embryo. The colors produced by aldehydes differed from the black reaction produced by peroxidase.

It is suggested that some of the flavor changes commonly encountered as a result of delay before commercial freezing of peas may be due to natural processes of tissue resorption and accompanying changes in cellular components.

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A COMPARISON OF STRAINS OF STAPHYLOCOCCI ISOLATED FROM FOODS

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At the present time, the staphylococcus food poisoning problem suffers from very obvious limitations in technique. Studies which will resolve many of these difficulties are undoubtedly in progress at various places. Until such major contributions can be made, there seems little use in adding to the already complicated picture, except that reports of work done may save time by preventing unnecessary repetitions. On such a basis the following comparison of the reactions of the staphylococci recovered both from foods known to have caused food poisoning and from foods not known to have been implicated in food intoxications, is offered.

The collection for analysis of the samples of foods not known to have caused food poisoning has been previously described, Hussemann and Tanner (1947). Of the strains of staphylococci recovered from such sources, 40 were chosen randomly for further study. For comparison, 28 strains of staphylococci which had been accepted as the causative agent in food poisoning outbreaks were secured.[3] All strains from both sources were submitted to selected tests which have been suggested as means of separating the enterotoxigenic strains from the non-enterotoxin formers. Techniques employed were the Stone (1935, 1936) reaction, the series of tests outlined by Chapman, Lieb, and Curcio (1937), the use of 7.5 per cent sodium chloride phenol red mannitol agar, Chapman (1945), and a very limited number of strains (seven from each group) were examined, using a kitten injection method. For the latter a crude filtrate was employed in which no attempt was made to destroy the alpha and beta toxins. Aside from this one rather major difference, the techniques of the kitten tests were those of Dolman and Wilson (1940). At least two kittens were used to test each filtrate examined.

Table 1 shows the results of the comparison made for certain biochemical reactions on strains of staphylococci recovered from foods not known to have caused food intoxications and strains recovered from foods which apparently had caused food poisonings. While the number of strains in both groups was very limited certain differences seemed to be indicated. Points of greatest divergence occurred in coagulase formation, in fermentation of mannitol with the formation of acid, and in degree and number of pigment formers.

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[3] Dr. C. E. Dolman, Dr. Lloyd Jensen, and Dr. R. V. Stone very generously contributed strains of organisms for this study.

Numerous laboratories have reported the number or percentage of staphylococci which were coagulase positive when a series of strains from a given source were tested. To choose only a few examples, Fisher (1936) reported that 26 of 34 strains having for their origin sties, bed-sores, blepharitis, etc., were coagulase positive. Hallman (1937) found that 68.7 per cent of 355 strains from the anterior nares of persons without evidence of upper respiratory infection was coagulase positive. In the studies of Fairbrother (1940), 124 of 127 strains of staphylococci from active infections produced coagulase, and 25 of 122 strains from the nasal and pharyngeal mucosa and urine of healthy individuals and from air contamination were coagulase positive. Christie and Keogh (1940) demonstrated coagulase formation in 70 per cent of the strains of human origin. Examination for the development of enterotoxin was not made in any of the aforementioned work.

TABLE 1

Comparison of Certain Biochemical Reactions Exhibited by Staphylococci Recovered From Foods not Known to Have Caused Food Poisoning With Those of Apparent Food Poisoning Strains

Techniques employed	40 strains of staphylococci from foods not known to have produced food poisoning	28 strains of staphylococci from foods incriminated in food-poisoning outbreaks
	pct.	*pct.*
Pigment formation on Loeffler's medium	White 32.5	White 3.6
	Cream 37.5	Cream 17.8
	Yellow 22.5	Yellow 3.6
	Orange 7.5	Orange 75.0
Hemolysis on rabbit blood agar	90.0 positive	96.4 positive
Coagulase (rabbit plasma)	2.5 positive	96.4 positive
Acid formation on phenol red mannitol agar	75.0 positive	100.0 positive
Growth on bromthymol blue agar	90.0 positive	96.4 positive
Growth on 7.5 per cent Na Cl phenol red mannitol agar	97.5 positive	96.4 positive
Stone's reaction	80.0 positive	75.0 positive

In the studies here reported, when the strains of staphylococci recovered from foods not known to have caused food poisoning were considered, the percentage which was coagulase positive was found to be lower than has commonly been reported. Those strains apparently enterotoxigenic showed the percentage of coagulase positive organisms to be in the range of that demonstrated in strains from other pathogenic sources. It may have been, of course, that had another of the techniques recommended been applied in the performance of the coagulase test, both the percentages positive in each group and the relationship of the groups to each other may have been different. It appeared here, as elsewhere, that most enterotoxin formers were coagulase positive strains. The actual percentage of coagulase formers which are enterotoxigenic has not been determined, so far as is known.

The fermentation of mannitol with the formation of acid as a criterion for potentially pathogenic staphylococci was first suggested by Gordon in 1905. In the present study, a difference of 25 per cent was found between

numbers of organisms fermenting mannitol with the formation of acid, in the group which had as its origin food which caused poisoning, and those from foods not known to have caused food intoxications. This would indicate there may be a differential value to the test. Hallman (1937) found a correlation of 90.97 per cent between coagulase positive organisms and mannitol positive staphylococci when 487 strains were tested. She emphasized that there was quite a difference in the rate at which the different strains of staphylococci fermented mannitol. Gwatkin (1937) stated that the mannitol fermentation appeared to have some practical value in forming an opinion as to the possible pathogenicity of the organisms. Chapman and co-workers (1938) pointed out that from their results it appeared that mannitol-negative strains were not likely to be pathogenic, but that the reverse did not necessarily hold true. Plastridge and co-workers (1939), working with strains of staphylococci found in milk, concluded that the ability to ferment mannitol (or lactose) usually indicated some degree of virulence, but that the ability to act upon these two substances is not limited to cultures of high virulence. Christie and Keogh (1940) found that errors which might arise from relying on mannitol fermentation as an indication of pathogenicity could be halved if the results with trehalose and mannose were considered along with those of mannitol. However, Kupchik (1937) presented evidence that there seemed to be no correlation between fermentation reactions, including mannitol, and the ability of the organism to form enterotoxin. This viewpoint is not entirely corroborated here. Judged on the basis of mannitol fermentation with the production of acid, the strains of staphylococci collected from foods which apparently did not cause food poisoning would potentially be much more dangerous than when judged by the coagulase standard.

Although pigmentation has not uniformly been associated with pathogenicity, suggestions of correlation do exist. Hallman (1937) determined that 81 per cent of orange staphylococci were pathogenic by the coagulase test, whereas only 17 per cent of the yellow and none of the white ones were pathogenic. She concluded, however, that since color range was almost identical for the pathogenic and non-pathogenic groups, not too much reliance should be placed on chromogenesis as a test for differentiation of pathogenic strains. Cowan (1938) and Fairbrother (1940), among others, indicated that both *aureus* and *albus* strains should be included in the pathogenic group and for the group they used the name *Staphylococcus pyogenes*. There seemed to be a correlation of pigment formation and food-poisoning activity, if the outbreaks for 1940 to 1945 were considered. By far the greatest number of outbreaks were said to be due to *aureus*, when a species was given. In our work, all variations of color appeared in both groups. Hence, it would appear, as has been previously observed, that chromogenesis is of no value in determining potential food-poisoners.

A few comments on the Stone reaction seem pertinent. It can be seen (Table 1) that the percentages of strains of staphylococci yielding positive results in each of the two groups were not markedly different. The Stone test was applied, in addition, to 31 strains of staphylococci recovered from prepared foods and ingredients apparently not causing food poisoning but not included in the studies reported (Table 1), making in

all 71 strains of staphylococci from such sources. Twenty-eight strains of food-poisoning staphylococci were submitted to the Stone reaction. Results are illustrated (Figs. 1 and 2). It was possible to get all degrees of zoning in each series of organisms. Therefore, this would seem to confirm the

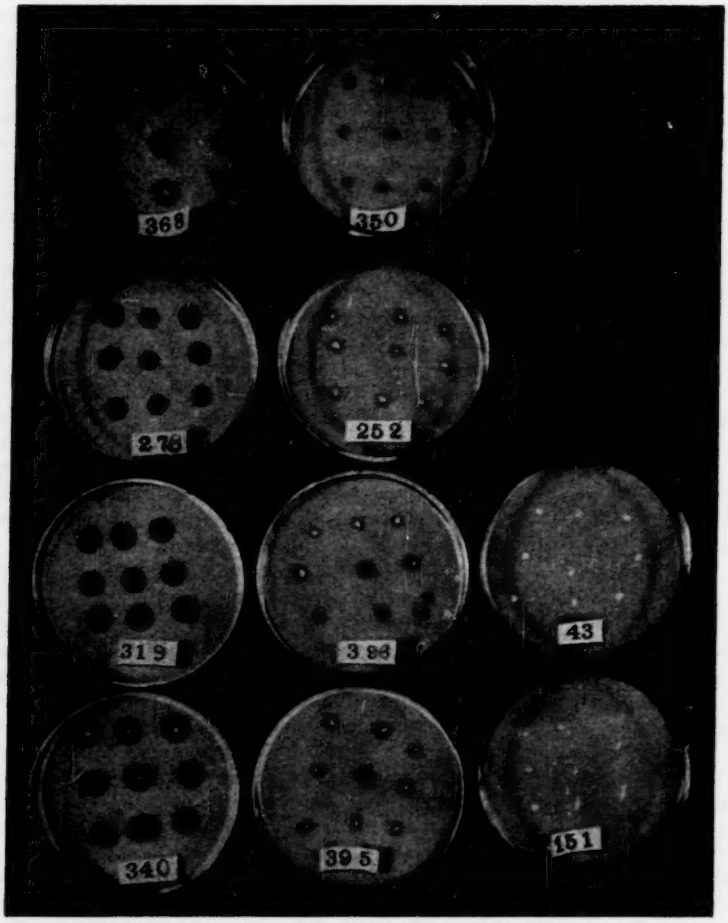

FIG. 1. Showing the reaction on Stone's medium of staphylococci recovered from food not known to have caused food poisoning.

works of other investigators, who found that this test was not a reliable index for food-poisoning staphylococci.

Great difficulty was experienced in procuring kittens, so that the work done using the Dolman test was of small value, because it was so limited. Toxins were made, using the Dolman method, except that alpha and beta

toxins were not neutralized or destroyed. More interest was felt here in the application of the findings to human feeding problems, and it seemed that in circumstances of food-poisoning outbreaks there would be no neutralization of the alpha and beta toxins. For this reason, cell-free filtrates *in toto* were injected intraperitoneally into kittens. Sixteen kittens were used, testing seven toxins produced by strains previously accepted as food-

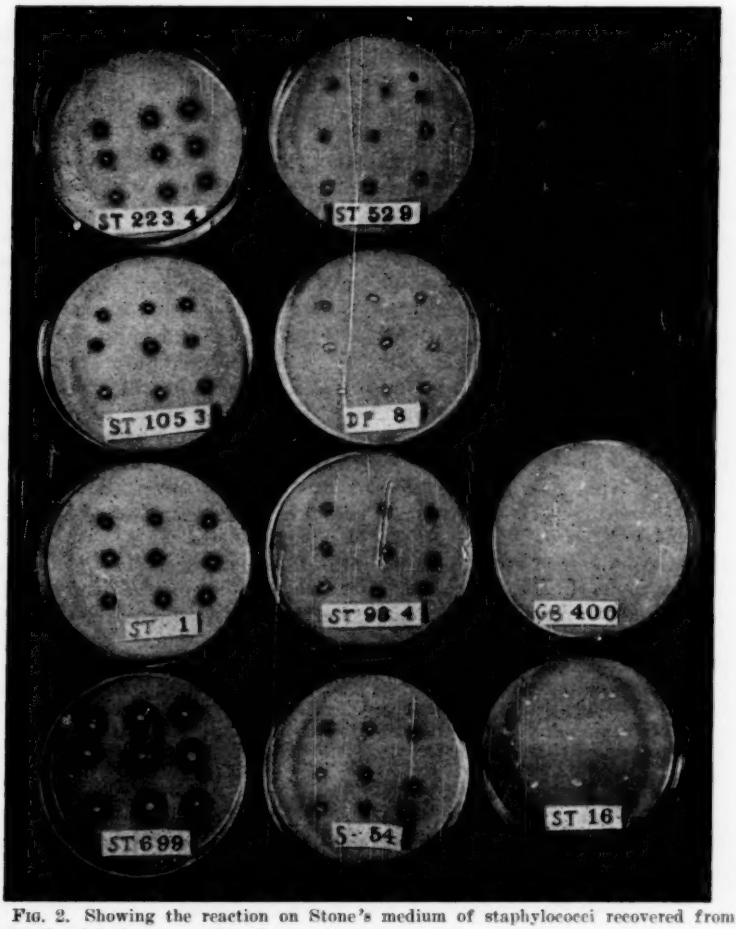

FIG. 2. Showing the reaction on Stone's medium of staphylococci recovered from food believed to have caused food poisoning.

poisoners. All kittens except three exhibited vomiting and diarrhea. Two different toxins accounted for the negative results, but in one case both kittens injected were negative, whereas for the other, three kittens were used and two were positive and only one was negative. Fourteen kittens

were used, testing toxins from seven strains of staphylococci obtained from foods which apparently did not cause food poisoning. In four cases both kittens vomited, in two cases one kitten was positive and one negative, and in one case both kittens negative. Two kittens were injected with uninoculated media and of these one gave a positive reaction and one a negative. As can be seen, no conclusion can be reached from this work with kittens, since one of the kittens injected with uninoculated media was positive.

It is obvious that results of these experiments, as well as the literature which preceded them, are confusing and far from sharply differentiated. Except for one strain (GB400), the food-poisoning group of strains had been carried for considerable lengths of time on synthetic media. The characteristics of this group as determined here may have been at variance with the reactions which might have been observed when the organisms were freshly isolated. Under the conditions of these experiments, however, none of the tests yet devised seemed to be wholly successful in separating the enterotoxin-forming from the non-enterotoxigenic staphylococci. Conditions necessary for the formation of enterotoxin are just beginning to be defined. Until techniques are available which permit toxin identification, results of finding staphylococci in even a food shown epidemiologically to be incriminated in a food poisoning, must remain in question. It has been demonstrated that staphylococci were harbored in foods which were eaten by large numbers of persons and with no untoward results, Hussemann and Tanner (1947). Perhaps some of those who in the past have isolated staphylococci from foods associated with food intoxications have picked an innocent bystander and allowed the real offender to escape. This may account for some of the lack of correlation observed here and elsewhere. Conversely, it might be possible to isolate a staphylococcus with all the characteristics usually associated with food-poisoning strains from a food which did not cause food poisoning. In one instance this was done during the course of this work. The implications are that our present-day tests are very inaccurate. Until definitive methods of identification of the enterotoxin are devised, all staphylococcus food-poisoning work is hampered.

NOTE: To promote uniformity in the papers reported from this thesis, the nomenclature for the group of organisms under discussion is retained as given in the fifth edition of Bergey's "Manual of Determinative Bacteriology." Recognition is given to the reclassification of this genus as outlined in the sixth edition of Bergey.

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FLUORINE CONTENT OF FUKIEN TEAS

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Smith, Lantz, and Smith (1931) found that fluorine was the causal agent of mottled enamel, a defect of human teeth. Since then much interest has been manifested in the detection of this element in food materials and drinking waters. Mottled teeth have been found endemic over certain areas in practically every country. Smith (1935) later found that drinking waters containing more than 0.9 part per million of fluorine caused mottling of enamel in human teeth. One of the writers (1936) has found three villages in the vicinity of Foochow where mottled teeth occurred, and examination of their drinking waters revealed a fluorine content of from 0.7 to 7 parts per million. Reid (1936) analyzed a number of Chinese food materials and found that teas are particularly high in their fluorine content. He also found that between 81 and 96 per cent of the fluorine in tea leaves could be extracted in a two per cent infusion. Since tea drinking is such a universal and common practice in China, Reid's findings are of much practical interest to nutritional chemists, as well as to tea manufacturers.

In view of this, the present investigation was undertaken with an aim of finding out the causal factors of the high fluorine content. Results of analyses of 30 samples of tea from various parts of Fukien (Tables 1 and 2) indicated high contents of fluorine. The price of a sample of tea seemed to serve roughly as an index of its fluorine content, cheap teas containing more fluorine than those of higher prices. As to its causal factors, this may suggest contamination for cheap teas are much more carelessly handled, giving ample chances for contamination by dirts and other foreign substances. To obtain further information along this line of reasoning, samples of low-grade tea, seeds, twigs, tea dusts, as well as sweepings from the floor of the manufacturing rooms, were analyzed. But results indicated to the contrary, for seeds and twigs contained less fluorine than the leaves of cheap tea, and tea dust contained more fluorine than the sweepings from the floor; so contamination as the cause of high fluorine seemed improbable.

Since the high content of fluorine was shown not to be due to contamination, it might have been caused by absorption from the soil in which the tea plant was cultivated. Six specimens of soils from Bohea Mountain and Chi-Shih, as well as teas from plants grown in these soils, were analyzed, but no correlation could be drawn from these results (Table 2). Tea leaves from soils of high fluorine content may contain even less fluorine than those from soils of lower fluorine content. This indicates that each variety of tea plant has its peculiar power to absorb a certain amount of fluorine from the soil, and that the concentrations of fluorine in the soils have all been above the level for maximum absorption.

TABLE 1

Fluorine Contents of Teas From Different Parts of Fukien

Type of tea and location	Fluorine in mg. per 100 g. dry tea
Green tea of Ling-Teh	
Average result of 3 samples	15.5
Green tea of Fu-an	
Average result of 4 samples	21.9
Green tea of Lo-Yuan	
Average result of 2 samples	10.3
Green tea of Fu-Ting	
Average result of 2 samples	19.9
Green tea of Shaowu	17.0
Oolong tea of Bohea Mountain, Chung-An	
Average result of 10 samples	19.1
Black tea of Chung-An	
Average result of 2 samples	21.0
Tea plant seeds	15.3
Twigs from tea	16.0
Low-grade fragments of tea	26.8
Tea dust	25.2
Tea dust from floor	17.9

Among other possible factors that may influence the fluorine content of a tea, the following may be mentioned: (a) difference in the power of absorption of tea plants, (b) age of the tea leaves, and (c) the influence of manufacturing processes. In order to study these factors free from the effects of unknown variables, three series of samples of tea were made from three batches of fresh leaves by expert workmen under our own supervision. From results tabulated (Table 3) it is clearly shown that Shui-sen leaves, which were generally considered superior in quality, contained less fluorine than the ordinary leaves of Batch 1. Shui-sen leaves are most abundantly cultivated at Bohea Mountain, Chung-An, for the manufacture of oolong tea, a highly priced and much valued tea. The

TABLE 2

Fluorine Contents of Tea and of Soils of Bohea Mountain, Chung-An.

Name of tea	Price[1]	Fluorine in tea, mg./100 g. dry tea	Location tea leaves and soils obtained	Fluorine in soil mg./100 g. dry soil
Oolong Tea:		*pct.*		
Shui-sen	6	5.7	.....	.....
Tang-chun	8	14.2	Pi-Shih Yen	29.9
Oolong	6	12.0	Ku-Tze-Tai-Men Yen	16.1
Tao-Jen	6	14.4	Mi-Tao Yen	13.7
Chi-Lan	6	7.2	Ching-Yuan Yen	8.6
Chi-Chun	5	21.4	.....	.....
Min-Chun	3	17.1	Pi-Shih Yen	13.8
Pei-Chai	1	30.5	.....	.....
Black Tea:				
Gongou	3	35.5	Chi-Shih	27.0
Green Tea:				
Fang-Lung-Ching	3	32.7	Chi-Shih	27.0

[1] Prices are in dollars per 500 g. as quoted by tea manufacturer, June, 1940.

Shui-sen leaves of Batch 2 came from Yun-Ping San, Shao-Wu, and were cultivated for the same purpose. Teas, green, black, or oolong, made from Shui-sen leaves, contained 10.1 to 10.6 mg. of fluorine per 100 gm. of dry leaves; while teas made from ordinary leaves, under identical conditions, contained 13.1 to 13.7 mg. of fluorine. This difference must thus be due to the tea plant itself.

TABLE 3

Fluorine Contents of Different Kinds of Tea From Same Batch of Fresh Leaves

Kind of tea	Dry product	Fluorine, mg. per 100 gm. on the basis of		Pct. fluorine extracted in a two per cent infusion
		Dry tea	Fresh leaves	
Batch 1 (Ordinary leaves)				
Fresh leaves, dried	38.12	13.00	5.0	34.6
Green tea	35.81	13.60	4.9	42.5
Lung-Ching (green tea)	35.50	13.60	4.7	42.5
Oolong (semi-fermented)	35.04	13.10	4.6	49.4
Black tea:				
After withering	36.27	10.69	3.9	36.9
After rolling	36.45	11.90	4.3	45.2
After fermenting				
2½ hours	36.99	12.30	4.6	46.6
3½ hours	36.87	13.00	4.8	44.7
4½ hours	36.42	13.70	5.0	51.0
Batch 2 (Shui-Sen leaves)				
Fresh leaves, dried	33.43	11.00	3.7	29.1
Green tea	33.03	10.60	3.5	40.6
Oolong:				
After fermentation	32.39	10.10	3.4	40.0
Finished tea	29.38	10.10	3.2	43.1
Black tea	37.60	10.50	4.0	50.5

Black tea was fermented; oolong tea, semi-fermented; while in the manufacture of green tea, fermentation was cautiously prevented; hence the processes of manufacturing each of them must necessarily differ widely. On account of the fact that the weight of dry product obtained from a fixed weight of fresh leaves varied slightly with the kind of tea prepared, which with Shui-sen leaves varied from 29.38 per cent for oolong to 33.03 per cent for green tea and 37.60 per cent for black tea, results of analyses were made more comparable by recalculating on the basis of fresh leaves (see Column 3, Table 3). Teas, whether green, oolong, or black, or whether made from Shui-sen or ordinary leaves, contained practically the same amount of fluorine as the fresh leaves from which they were made. This indicated no change had taken place with fluorine, so far as the total quantity of the element was concerned, during the various processes of manufacturing, such as withering, rolling, firing, fermentation, etc.

In the manufacture of black tea of Batch 1, the fluorine content of leaves after withering was 3.88 mg. per 100 gm. of fresh leaves, while that of the fresh leaves and of the finished tea were 4.97 and 4.95 respectively. This seems unreasonable, but no satisfactory explanation could be

offered, except that it was due to some error made in the determination. Data (Table 4) show clearly the effect of age of tea leaves on fluorine content; bulbs and first leaves contained the least amount of fluorine, while older leaves contained considerably more. This also furnished an explanation as to why the more expensive teas were lower in fluorine, for they were generally made from the younger leaves.

TABLE 4

Fluorine Contents of Black Tea From Leaves of Different Ages

Black tea from	Fluorine, mg. per 100 g. dry tea	Pct. fluorine extracted in a two per cent infusion
Bulbs and first leaves	11.3	47.8
Second leaves	12.4	51.6
Third leaves	14.2	52.8
Fourth leaves	16.4	.....

As Reid (1936) had pointed out that the amount of fluorine extracted in the infusion was more important than the total amount in the tea leaves, this point was carefully studied. The amount of fluorine that may be extracted in a two per cent infusion was found to be between 40.6 and 42.5 per cent for green tea; between 43.1 and 49.4 per cent for oolong tea; and between 50.5 and 51.0 per cent for black tea. These values are considerably lower than those found by Reid (1936), which were between 81 and 96 per cent. It may also be pointed out from the data on leaves of Batch 1 (Table 3) that the amount of fluorine extracted was lowest in the fresh leaves, namely 34.6 per cent in oolong tea, 49.4 per cent in green tea, and 51.0 per cent in black tea. These facts indicated two things, namely, (a) that in the process of manufacture of green tea, which, in brief, consisted of roasting the leaves immediately after withering in a highly heated iron pan, so as to avoid fermentation, followed by rolling and drying, etc., the fluorine was thus rendered more soluble in water due to the breaking of the tissue walls and exposure of the contents more readily to the action of the solvent; (b) that fermentation which occurred to a limited extent in the manufacture of oolong tea and to a much greater extent in the manufacture of black tea, had further rendered the fluorine more soluble.

Thus, the amount of fluorine extracted is in the order of the extent of fermentation that has been allowed to take place, being highest in black tea and lowest in green. Oolong tea, being semi-fermented, has a value intermediate between the two extremes. Another interesting fact may be noticed by comparing the amount of fluorine extracted from the fresh leaves, green tea, oolong tea, and black tea made from the Shui-sen leaves of Batch 2 with those of Batch 1. It will be seen that in every case the amount of fluorine extracted from teas made from Shui-sen leaves is lower than that of the corresponding kind of tea made from the ordinary leaves of Batch 1, thus indicating once more the superior quality of the Shui-sen

leaves. Age of the leaves exerts considerable influence on the extractability of the fluorine; the amount increased from 47.8 per cent for the bulbs and first leaves, to 51.6 per cent for the second leaves, and 52.8 per cent for the third leaves.

EXPERIMENTAL PROCEDURE

Method of Analysis: For the determination of fluorine in tea, the samples were weighed out and ashed at a temperature not exceeding 400 to 450°C.(752 to 815°F.) in the presence of sodium hydroxide, magnesium nitrate, and calcium acetate. The ash was then transferred to a 250-c.c. distilling flask, 50 c.c. concentrated sulphuric acid added, and the fluorine distilled over, according to the method described by Boruff and Abbot (1933). In the subsequent determination of the fluorine in the distillate, Sanchis' (1934) colorimetric method was employed.

For the determination of fluorine in soils, Steinkoenig's (1919) modification of the Merwin method was followed.

For the determination of the extractability of the fluorine, infusions of different samples of tea were made by boiling two grams of the sample in 100 c.c. of water for five minutes and fluorine determined according to the method described above.

Tea Samples: Teas of various grades from several parts of Fukien province and, in a few cases with the soils in which the tea plants were cultivated, were obtained and analyzed for their fluorine contents. In order to find out the effect of the manufacturing processes on the fluorine content, two batches of fresh leaves were obtained and from each of these green, black, and oolong tea were made in the University tea laboratory, by expert workmen, under our own directions. The finished teas, as well as samples from various intermediate stages, were analyzed for fluorine. In a third batch, leaves from each twig were separated and sorted into four groups: the bulb and the leaf immediately next to it, the second leaves, the third leaves, and the fourth leaves. Each group of leaves was separately made into black tea and its fluorine content determined.

Results of analyses of various samples of tea are given (Tables 1 to 4).

SUMMARY

A study was made on the fluorine contents of Fukien teas and analytical results indicated the amount ranged from 5.7 to 35.5 mg. per 100 grams of dry tea.

The high content of fluorine was found not to be due to contamination, nor to the high fluorine content of the soil in which the tea plant was cultivated. Differences in the methods of manufacture had no effect on the fluorine content of the final products.

Different varieties of tea plants have different powers to absorb fluorine from the soil. Of the two varieties of tea plants studied, Shui-Sen leaves possessed the lower fluorine content. Age of the tea leaves exerted an important influence on the fluorine content, the older leaves containing considerably more fluorine than the younger.

The amount of fluorine that may be extracted in a two per cent infusion varies from 29.1 per cent for fresh leaves to 50.5 per cent for black tea. The process of roasting and rolling rendered the fluorine more soluble,

hence the amount extracted increased in green tea. Fermentation further increased the extractability of the fluorine; thus the amount extracted was the highest in black tea, which was fermented, less in the semi-fermented oolong tea, and least in the unfermented green tea. The extractability of fluorine was also increased with age of the leaves.

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RETENTION OF B VITAMINS IN BEEF AND VEAL AFTER HOME CANNING AND STORAGE

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Cover, Turk, and Kerns (1943) reported a safe method of home canning meat which required shorter processing times than were usually recommended at that time. In view of the great emphasis being placed on the nutritive value of food, it seemed timely to determine the retention of the B vitamins in meat canned by this method. This report on beef and veal will be followed later by one on pork.

EXPERIMENTAL PROCEDURE

Changes in Procedure: It seemed desirable to make certain changes in the procedure of Cover, Turk, and Kerns. Their containers were filled rounded-full to allow for shrinkage caused by steaming. Later it was observed that many housewives prefer to allow some head-space in order to retain the juices in the containers. Accordingly, preliminary trials were made to determine the definite weight of meat for each size of container which would give a container full of meat and broth but little or no evidence in the cooker or on the mouth of the container that juice had overflowed. This was secured with 530 grams (1.2 lb.) in No. 2 cans, 920 grams (2.0 lb.) in No. 3 cans, 420 grams (0.9 lb.) in pint jars, and 800 grams (1.8 lb.) in quart jars. These weights of meat were used in the retention studies.

Another change in the procedure of Cover, Turk, and Kerns was the amount of water in the cooker during steaming. It was found that water to within two inches of the top of the containers boiled rapidly enough to splash into the containers and cause juice to overflow. In the present study the water level was held to or below the half-way point on the lower layer of containers. The heat was adjusted so that only a small thread of steam came from the petcock. Thermometer readings showed a uniform temperature of 100°C.(212°F.) under these conditions.

Details of Canning Procedure: A 25-quart aluminum pressure cooker equipped with a thermometer, as well as a pressure gauge, was used. It held 16 No. 2 cans, 10 No. 3 cans, 16 pint jars in two layers each, and seven quart jars in one layer. During steaming the containers in the upper layer were marked with string. After steaming the containers in the upper layer were sealed before the lower layer was removed. Then, while the lower layer was being sealed, what had been the upper layer during steaming was placed in the hot cooker as the lower layer, for processing. This order allowed the containers as little chance as possible for cooling and was desirable because the processing time was determined for a high starting temperature. (In the test for veal, only one layer of pint jars was used because of the possibility of damage to the war-time

type closures of the jars in the bottom layer.) The petcock was closed after steam had escaped in a heavy stream for seven minutes. Thermometer readings showed that the cooker temperature was 105 to 106°C. (221 to 223°F.) for several minutes before the petcock was closed. After the processing temperature at 121°C. (15 pounds) had been reached, the thermometer was watched constantly. In no test did the processing temperature vary more than ± 1°C.

Steaming and processing times for the different sizes of containers were as follows:

Container	Steaming time	Processing time
	min.	*min.*
No. 2 cans	60	60
No. 3 cans	90	85
Pint jars	60	60
Quart jars	80	75

The cans were cooled in running water, the jars in air. After cooling, half the containers were analyzed immediately for their vitamin content.

Storage: The remaining containers were stored at summer room temperature for three months, then analyzed for vitamin content. During the summer the storage place was a dark closet in the laboratory, where the temperatures were from 95 to 98°F. In the winter these temperatures were maintained in a special steam-heated storage room.

Sampling: The study was planned to include two replications of beef, but veal only was available for the second replication.

In the test with beef, enough meat for an entire cooker full of containers was ground, mixed, reground, and remixed. A definite weight of meat was placed in each container. Only one raw sample was used, but it was analyzed in triplicate. Each container was analyzed separately.

In the test with veal, the meat was mixed, ground in large batches, then remixed in small batches. Each batch was used to fill a number of containers, with enough left over for two raw samples. Each raw sample and each container were analyzed in duplicate, using two separate standard curves for the microbiological methods. (Separate standard curves are necessary to obtain results which are independent enough to serve as duplicates.)

For both beef and veal, the meat and broth from one container were mixed in a Waring blendor and a sample of the blend taken for analysis. Before blending, the weight of the contents of each container was secured, except in a few instances.

Vitamin Methods: Thiamine, riboflavin, pantothenic acid, and niacin were determined on the papain-takadiastase extracts from which the fat had been removed with chloroform. Thiamine was determined by the thiochrome procedure as modified by Cover, McLaren, and Pearson (1944). For the other three vitamins the extract was further purified by precipitation of the proteins at pH 6.8 to 7.0. The following microbiological methods were used: Riboflavin, Snell and Strong (1939); niacin, Snell and Wright (1941); pantothenic acid, Strong *et al.* (1941).

Retentions were calculated on the dry fat-free basis. Because of incomplete data on weight of contents after treatment, retentions could not be based on total vitamin content per can before and after treatment.

DATA AND DISCUSSION

Retention After Canning: During home canning of beef and veal at 15 pounds' pressure, 121°C.(250°F.), the thiamine retention in pint and quart jars and in No. 2 and No. 3 tin cans was about 35 per cent or less (Table 1). Reedman and Buckby (1943), Rice and Robinson (1944), and

TABLE 1

Retention of B Vitamins in Meat After Canning[1]

Container	Thiamine		Riboflavin		Pantothenic acid		Niacin	
	Beef	Veal	Beef	Veal	Beef	Veal	Beef	Veal
	pct.	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*
No. 2 cans								
Range	27-39	21-27	99-112	87-106	79-88	66-86	92-107	75-96
Mean	35	23	105	97	84	71	97	85
No. of containers	(8)	(8)	(8)	(8)	(6)	(8)	(8)	(8)
No. 3 cans								
Range	21-25	11-22	103-107	93-98	78-84	56-66	90-95	93-99
Mean	22	16	105	95	80	61	92	97
No. of containers	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
Pint jars								
Range	29-42	22-30	83-97	91-97	70-82	56-71	95-111	82-100
Mean	35	28	91	93	73	64	101	91
No. of containers	(8)	(4)[2]	(8)	(4)[2]	(8)	(4)[2]	(8)	(4)[2]
Quart jars								
Range	15-28	20-23	98-103	90-95	.....	65-72	89-109	86-95
Mean	22	21	101	92	.....	68	98	90
No. of containers	(4)	(4)	(4)	(4)	.....	(4)	(4)	(4)

[1] Based on dry fat-free values of canned and raw meat. [2] Only one layer of pint jars was processed in this test with veal.

Greenwood *et al.* (1944) reported thiamine retentions in ground pork mixtures processed under commercial conditions. Their retentions in 12-ounce cans varied from 67 to 76 per cent; in 2½-pound cans from 46 to 60 per cent; and in six-pound cans from 38 to 53 per cent. All thiamine retentions obtained under commercial conditions were considerably higher than those obtained under home conditions. However, home and commercial practices differ in can sizes, processing temperatures, and processing times. Greenwood *et al.* gave the F_0 values of their heat treatments; these were considerably lower than those obtained in the processes recommended for home canning. The higher heat treatments used in home canning are no doubt the reason for the greater loss of thiamine during home canning than during commercial canning.

During home canning of beef and veal some pantothenic acid was lost (about 30 per cent), but losses of niacin and riboflavin appeared to be negligible. Under commercial conditions, Rice and Robinson (1944) and Greenwood *et al.* (1944) reported negligible losses of niacin and riboflavin, but the former obtained some loss of pantothenic acid (24 per cent). For these three vitamins, losses in beef and veal canned under home conditions appear to agree well with those obtained in pork canned under commercial conditions.

Loss After Storage: After storage for three months at summer temperatures, 95 to 98°F., the thiamine content of the home canned meat was still further reduced (Table 2). Feaster *et al.* (1946) studied the effect of storage of canned pork luncheon meat at various temperatures. Their graphs indicated a loss of about 50 per cent of the thiamine after three months at 98°F.

TABLE 2

Loss of B Vitamins During Storage of Canned Meat [1]

Container	Thiamine		Riboflavin		Pantothenic acid		Niacin	
	Beef	Veal	Beef	Veal	Beef	Veal	Beef	Veal
	pct.	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*
No. 2 cans	89	5	21	+23	11	+ 6	1	+ 5
No. 3 cans	37	48	28	+ 6	+22	+12	+14	+21
Pint jars	82	37	15	+42	13	3	2	+11
Quart jars	77	75	5	+16	.....	+32	17	+12

[1] Calculated from average contents on dry fat-free basis of freshly canned and stored canned meat.

After storage of the home-canned meat, retentions of pantothenic acid, niacin, and riboflavin varied considerably among the various containers, but little if any further loss of these vitamins is indicated. Feaster *et al.* (1946), reported insignificant losses of pantothenic acid, niacin, and riboflavin after storage for 12 months at 98°F. Analyses of the stored samples of home-canned meat were not made at the same time as the raw and canned samples. At the time these tests were made, it was not understood that frozen samples could be used as controls for the stored canned meat and so none was provided. Thus, variations in technique, while not obvious to the analyst, may have influenced the values for retention after storage.

Vitamin Content: The average vitamin content of the meat as raw, canned, and after storage is given (Table 3). These values are on the moist basis. Since raw beef is one of the better sources of niacin and little or none is lost during canning or storage, meat canned by the method developed at the Texas Station should be an excellent source of niacin. A No. 2 can of meat makes four generous servings. Such a serving of beef or veal may supply between one-third and one-half of the niacin recommended for one day. On the other hand, a serving of home-canned beef or veal would supply only minor amounts (less than five per cent) of the thiamine recommended daily, and if the canned meat were kept over the summer without benefit of cool storage, the stored meat might contain less thiamine. A serving of home-canned meat may supply from 10 to 15 per cent of the recommended daily allowance of riboflavin.

SUMMARY

Beef and veal were canned in No. 2 and No. 3 tin cans and in pint and quart glass jars. Processing was done at 15 pounds' pressure for the times found by Cover, Turk, and Kerns to protect against spoilage by *Clostridium botulinum*. Analyses for thiamine, pantothenic acid, niacin, and riboflavin were made in duplicate on the mixture of meat and broth in each container and on the meat in each raw sample. Retentions were

TABLE 3

Average Content of B-Vitamins in Beef and Veal, Moist Basis

State of sample	Thiamine		Riboflavin		Pantothenic acid		Niacin	
	Beef	Veal	Beef	Veal	Beef	Veal	Beef	Veal
	μg./gm.	μg./gm.	μg./gm.	μg./gm.	μg./gm.	μg./gm.	μg./gm.	μg./gm.
No. 2 cans								
Raw	1.73	1.28	2.14	1.45	3.77	4.38	39.74	50.47
Canned	0.63	0.31	2.33	1.50	3.24	3.30	40.41	46.23
Stored	0.07	0.29	1.82	1.84	2.85	3.47	40.45	48.25
No. 3 cans								
Raw	1.09	1.21	1.80	1.46	2.86	4.32	42.56	49.32
Canned	0.25	0.19	1.94	1.38	2.36	2.59	40.20	47.40
Stored	0.15	0.11	1.37	1.58	2.82	3.14	44.85	52.39
Pint jars								
Raw	1.73	1.30	2.14	1.26	3.77	3.62	39.74	44.82
Canned	0.61	0.40	1.99	1.31	2.92	2.57	42.91	45.13
Stored	0.11	0.25	1.68	1.85	2.43	2.47	42.00	49.60
Quart jars								
Raw	1.38	1.27	1.96	1.69	.....	3.29	59.12	50.11
Canned	0.32	0.29	2.06	1.70	.....	2.45	60.38	49.05
Stored	0.07	0.08	1.92	2.13	.....	3.50	50.53	59.56

calculated on the dry fat-free basis, immediately after canning, and after storage for three months at summer temperatures (95 to 98°F.).

After canning, retentions of thiamine were 35 per cent or less, considerably less than those obtained with pork luncheon meat under commercial conditions, where lower heat treatments were used. Pantothenic acid retention was about 70 per cent. Losses of niacin and riboflavin were negligible.

During storage further losses of thiamine were obtained, but little if any losses of pantothenic acid, niacin, or riboflavin occurred during storage.

The vitamin content of canned beef or veal is such that a generous serving may supply between one-third and one-half of the niacin and 10 to 15 per cent of the recommended riboflavin recommended for one day, but only minor amounts (less than five per cent) of the recommended thiamine.

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SEMI-MICRO METHOD FOR DETERMINING COAGULATION TEMPERATURE

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Bancroft and Rutzler (1931) reviewed the then-current postulates as to the nature of protein coagulation. They reached the conclusion, now generally accepted, that coagulation is a colloid phenomenon.

In heat coagulation of egg albumen there appear to be two consecutive changes. The first is the loss of water by the hydrophylic colloid—the egg white sol and gel constituents; the second, a gelatinous precipitation of the so-sensitized protein by the naturally occurring electrolytes in the dispersion medium. The question as to the factor which initiates the loss of water in the first step is still unsatisfactorily answered. In the opinion of some this change is strictly chemical, involving the protein only; others look upon it as a physical-chemical change, involving both proteins and constituents in the dispersion medium.

In any case, it is possible that coagulation data have been neglected as indirect evidence in establishing the nature of the primary change. Regardless of the method used, coagulation temperatures by different workers will vary, because they have duplicate samples only by accident. Egg albumen undergoes two changes, starting with the time the egg is laid: viscosity decreases and CO_2 is lost. The possible relation of the first change to the gelatinous precipitation is obvious; the loss of CO_2 starts a shifting in the total electrolyte equilibrium, including the change of carbonic acid to compounds which no longer control the acidity, as pH. Thus, critical temperatures reported have been specific to the samples.

A review of the current work of egg specialists failed to reveal a detailed description of a method giving sufficiently reproducible results. More work has been reported on coagulation of egg white than on any other single protein. Some reports simply give the time-temperature relation in coagulation of immersed shell eggs; other workers have broken the eggs into hot water and "poached" them. The water-jacket heating of albumen in a test tube provided with a thermometer or thermocouple has been employed by some.

The criteria of coagulation range from an initial cloudiness to a soft gel, or in one case, to a firm gel which is subject to syneresis when stirred. This lack of sharp definition of coagulation temperature further limits the significance of these reported data.

DETAILS OF THE SEMI-MICRO METHOD

The study here reported was not especially concerned with the coagulation temperature, *per se*, but rather with a method of determining the temperature, reproducibly, for different protein sols. Egg white, which supplies four readily separable layers of colloid materials, was used as the protein dispersion. The layers were separated with two pipettes of

different-sized bores and with forceps, as described by Romanoff (1943). For each test several eggs were broken and the whites separated. The composites of each layer from the several eggs were intimately mixed with mortar and pestle to break up physical gel or membrane structures, so that the samples tested would have greater uniformity.

The method devised is an adaptation of the technique officially standard for melting-point determinations of crystalline materials. Coagulation point tubes were made for this use by heating soft glass tubing of three mm. diameter and 2.25 mm. bore, and drawing it out to thin-walled tubes of approximately one mm. diameter, having bores of near-capillary size. The most convenient over-all length is five to six inches, with about one inch of the original size left at the top for handling and for drawing the albumen into the capillary tube.

TABLE 1

Reproducibility of Coagulation Temperatures by a Semi-Micro Method

(Colloid Sols: From the four layers of egg white)

	Outer thin	Middle thick	Inner thin	Inner thick
	A — B[1]	A — B[1]	A — B[1]	A — B[1]
Lot I (White shell)	°C.	°C.	°C.	°C.
1	63.5-66.0	63.5-67.0	64.0-67.0	64.0-67.5
2	63.5-65.5	63.5-67.0	64.0-67.0	64.0-67.5
3	63.5-65.5	63.5-66.5	64.0-67.0	64.5-67.5
Average	63.5-65.7	63.5-66.8	64.0-67.0	64.2-67.5
Lot II (Brown shell)				
1	63.0-65.5	63.5-66.0	64.0-66.0	63.5-66.0
2	63.5-65.5	63.5-66.0	64.0-66.0	64.0-66.5
3	63.0-65.5	63.5-66.0	63.5-65.5	63.5-66.5
Average	63.2-65.5	63.5-66.0	63.8-65.8	63.7-66.3

[1]A—first bluish opalescence; B—definite opacity.

After the tube is filled, the small end is sealed by touching it to the blue flame of a small alcohol lamp. Such heating results in a minimum of coagulation of albumen just above the seal.

Routine for making the tests is as follows:

The filled, sealed tube is bound to the bulb of a thermometer as in melting-point technique, the portion of the tube opposite the thermometer bulb being well above the tip containing albumen already coagulated during sealing. The thermometer, carrying the coagulation point tube, is then suspended in a one-liter beaker containing freshly boiled water, cooled to 40°C.(104°F.). Boiling is necessary to remove air which otherwise collects as bubbles on the tube, interfering with visibility of the albumen therein.

The beaker of water is placed on such a heating unit as will maintain an even rise in temperature of from 3 to 4°C. per minute, thus giving the necessary control to the time factor. In this laboratory, a six-inch element of an electric stove, set at "High," gave the necessary heat; a mechanical stirrer kept the water in motion throughout the heating.

For this work, the temperature was recorded at the point when the material in the tube took on a bluish opalescence (Column A, Table 1) and again when, after passing through a gradually increasing cloudiness, it reached an unmistakable opacity (Column B, Table 1).

Typical limits for the method described are given for two lots of eggs (Table 1).

DISCUSSION

No attempt is made at this time to determine the number of duplicate tests necessary to establish a coagulation temperature. The triplicate determinations for the four layers of Lots I and II provide eight sets of figures for comparison, with two different criteria considered in each case.

TABLE 2

Comparison of Coagulation Temperatures of Sols

(Representing eggs with different histories)

	Outer thin	Middle thick	Inner thin	Inner thick
	A — B[1]	A — B[1]	A — B[1]	A — B[1]
	°C.	°C.	°C.	°C.
Lot I (Average) (White shell)	63.5-65.7	63.5-66.8	64.0-67.0	64.2-67.5
Lot II (Average) (Brown shell)	63.2-65.5	63.5-66.0	63.8-65.8	63.7-66.3
Lot III (Brown shell)	63.0-66.0	63.0-65.5	63.0-66.0	63.5-66.0
Lot IV (White shell)	62.5-64.0	63.0-64.5	62.0-64.0	63.5-65.5

[1]A—first bluish opalescence; B—definite opacity.

By either criterion of judging coagulation, four out of the eight trials (Table 1) were identical in the triplicate determinations; the remaining four varied by 0.5°C. This difference is well within the limits obtained in this laboratory from the test tube method mentioned above. Tests on the same supplies of eggs by the test tube method always ran a few degrees lower than the measurements given (Table 1). This difference is attributed to the lag in heat penetration through the glass of the test tube and the egg white itself. In fact, results obtained by the test tube method could be varied more than 1.0°C. by the amount of stirring during the pre-coagulation period. The thin glass of the coagulation point tube and the thin layer of albumen in the semi-micro method minimize this source of error.

It is probable that each worker will need to establish the limits of error for equipment and technique used. For the time being, a variation of 0.5°C. is considered significant for work done in this laboratory.

It is known that coagulation temperatures of protein sols will reflect certain variables, such as pH, physical and chemical condition of the protein, and kind and amount of ions present in relation to the pH of the sol. The data (Table 2) suggest that this method will be of assistance in establishing a more exact relationship between some of these variables which are expected in egg whites from different sources and with different histories. Included in the four lots (Table 2) were eggs of at least two breeds and with four storage histories.

SUMMARY

A semi-micro method of testing the coagulation temperature of protein sols is described. Materials obtained from the four readily separable layers of egg white were used as test materials.

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STUDIES ON PRESERVATION OF SHELL EGGS BY COATING WITH PLASTICS

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Dipping hen's eggs in mineral oil or in oil emulsions is an effective method for retarding their losses of carbon dioxide and water vapor during subsequent storage, Romanoff and Yushok (1948). The ability of mineral oil to reduce the permeability of the intact eggshell and thus preserve the fresh quality of the egg contents has led to its extensive use in treating eggs destined for storage, in spite of several shortcomings of this treatment. Rosser (1942) observed that immersing eggs in oil prior to storage favors the growth of molds on the shell as well as in the egg. Our preliminary observations indicate, furthermore, that oil treatment does not prevent deterioration resulting from purely chemical changes in eggs whose shells are cracked or checked. A large opening in an eggshell cannot be sealed by mineral oil and presumably permits penetration of fungi and bacteria into the albumen and the yolk, where prolific growth can take place. During cold storage, a cracked egg promotes the growth of molds on the eggs surrounding it, since it not only supports the production of great numbers of spores but also increases the humidity of the air in its immediate vicinity.

For these reasons our investigations on preservatives were extended to the coating of intact and cracked eggs with a solid, continuous film which would be highly impermeable to carbon dioxide and water vapor and which would also present a mechanical barrier to microorganisms. In this connection, synthetic resins with thermoplastic properties were tested as sealing agents. The efficacy of various substances, when added to the resins to impart certain desirable physical properties to the plastic film, was also considered.

The ability of each plastic film to preserve eggs was evaluated by two measurements: (1) loss in weight of eggs, which is essentially the loss of water; (2) change in pH of the albumen, which is related to the escape of carbon dioxide. Further investigations into the prevention of mold growth on coated and untreated eggs will be discussed in a separate communication.

METHODS AND MATERIALS

Over 800 infertile eggs from White Leghorn hens were used. They were selected by candling within six hours after being laid and allowed to stand at room temperature for two hours. They were then immersed individually for a two-second period in the respective plastic solutions,[1]

[1] The authors wish to thank Bakelite Corporation, Catalin Corporation of America, and Hercules Powder Company for supplying samples of Vinylite polyvinyl acetate, Loabond polystyrene, and Parlon chlorinated rubber, respectively, from which the various solutions were prepared. They are also indebted to the Department of Chemical Engineering, Cornell University, for donating several plastic materials.

which were also at room temperature. After the excess coating solution had drained from the egg into the dipping beaker, the coated egg was allowed to dry thoroughly on a wire rack, the thin, firm prongs of which contacted the smallest possible surface of the egg. The drying time was about one minute or slightly longer, depending upon the nature of the solvent and the concentrations of synthetic resin and of modifying substances.

Each egg was then weighed to the nearest 0.1 gram and was stored in a forced-draft incubator in which a temperature of 37.5°C.(99.5°F.) and a relative humidity of approximately 35 per cent were maintained. After a 10-day storage period the weight of each egg was taken, and the pH of a five-ml. sample of its dense albumen measured by means of a Beckman laboratory model pH meter, using a glass electrode. The physical appearance of the albumen and yolk of each egg was observed and described.

Cracked eggs sealed with plastics were treated and stored under the same conditions as eggs with sound shells. A uniform crack on one side of each egg, extending from end to end, was made by striking each egg repeatedly with a sharp knife.

RESULTS AND DISCUSSION

A. *Unmodified Thermoplastic Resins*[2] *as Sealing Agents.*

In the literature surveyed, the only reference to the coating of eggs with a plastic material is made by Rosser and co-workers (1942), who tested polyvinyl alcohol and concluded that this water-soluble synthetic resin, when used alone, does not seal the egg perfectly and inhibits the growth of neither bacteria nor molds. Our data presented (Table 1) also indicate that a coating of polyvinyl alcohol is quite permeable to the passage of water and carbon dioxide and, therefore, has little preservative value.

Polystyrene, polyvinyl acetate, and chlorinated rubber were selected for our tests, because each absorbs very little moisture and is soluble in common organic solvents with low boiling points. Each of the three grades of polyvinyl acetate was much more effective than polyvinyl alcohol in preventing loss of water from the eggs. As the data (Table 1) indicate, during a 10-day storage period at 37.5°C., polyvinyl acetate which softens at 86.5°C.(188.6°F.) also prevented the pH of the albumen from rising above 8.0, although in untreated eggs it increased to 9.4. Since the increase in the pH of the albumen is accounted for by the loss of carbon dioxide, it appears that polyvinyl acetate is less permeable to carbon dioxide than unplasticized polystyrene. Polyvinyl acetate, however, is more permeable to water vapor.

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[2] Most commercial plastics are variable mixtures of compounds formulated to meet certain conditions of use. The basic synthetic materials employed in making plastics may be divided into two groups: thermoplastic and thermosetting resins, Winding and Hasche (1947). Thermoplastic resins in solution at room temperature may be used to form films, which soften when heat is applied. These properties of thermoplastic resins are desirable for the development of a simple method for coating eggs with a film which will not cause the treated eggs to crack when they are immersed in hot water. Thermosetting plastics were not tested as egg-coating agents because they are usually formed as finished non-softening products through the application of heat and pressure.

Our data indicate that polyvinyl acetate of a higher molecular weight (softening point—86.5°C.) is more impermeable to moisture and carbon dioxide than those varieties which have lower molecular weights and soften at lower temperatures. This higher impermeability is partly due to the fact that the more heat-resistant material (dissolved in ethyl alcohol) has a higher viscosity, and a thicker film is deposited on the egg. In view of the practical objectives of this study and the fact that in some cases the pore spaces of the shell are penetrated by sealing material, it was not deemed desirable to study the water and carbon dioxide perme-

TABLE 1

Effect of Synthetic Resin Films on Loss in Weight of Eggs and on Alkalinity of Albumen[1]

Synthetic resin			Solvent	Relative loss in weight of eggs[2]	Alkalinity of albumen[3]
Type	Description	Concentration by weight			
		pct.		*pct.*	*pH*
Polyvinyl alcohol	Medium viscosity	4.0	Water	67	9.2
Polyvinyl acetate	Softening point, 66°C.	10.0	Ethyl alcohol, 95 pct.	36	8.6
Polyvinyl acetate	Softening point, 77°C.	10.0	Ethyl alcohol, 95 pct.	32	8.5
Polyvinyl acetate	Softening point, 86.5°C.	10.0	Ethyl alcohol, 95 pct.	29	8.0
Polystyrene	Loabond emulsion, 40 pct. solids	.....	Water	38	9.1
Polystyrene	Molecular weight, 30,000	10.0	Ethyl acetate	26	8.9
Polystyrene	Molecular weight, 102,000	5.0	Ethyl acetate	22	8.9
Chlorinated rubber	Viscosity, 125 centipoises	10.0	Carbon tetrachloride	21	8.7
Chlorinated rubber	Viscosity, 125 centipoises	10.0	Ethyl acetate	17	8.3
Mineral-oil-dipped eggs				12	7.8
Untreated eggs				100	9.4

[1] Eggs held for a 10-day period at 37.5°C.(99.5°F.) and about 35 per cent relative humidity. [2] The percentage loss in weight of each set of treated eggs was divided by the percentage loss in weight of untreated eggs, then multiplied by 100 to give this index. [3] The pH of albumen from strictly fresh eggs was about 7.7.

ability in relation to the thickness of the film. A standard plastic concentration of 10 per cent by weight was chosen in most cases, because at this strength a thin, uniform, rapid-drying film was deposited. The various plastics and modifying substances were compared under as natural conditions of use as possible in order to detect any undesirable effects of the plastic coating on the egg.

Dipping eggs in a water emulsion of polystyrene less effectively decreased the permeability of the eggshell to water and carbon dioxide than immersing them in an ethyl acetate solution of this plastic. More than five minutes were required to dry a polystyrene film applied from water emulsion. The use of heat to hasten the drying time is undesirable, because the gases driven out of the expanding egg contents tend to form

bubbles while the plastic is solidifying. Further studies of water emulsions were not pursued for the following reasons: (1) Plastic-in-water emulsions usually can be expected to contain some hydrophilic constituent which would increase the water vapor permeability of a plastic film. (2) The relatively long drying time required to evaporate water at room temperature would necessitate the use of rather complex equipment for the commercial plastic coating of eggs.

As shown by the data (Table 1), 10 per cent chlorinated rubber dissolved in ethyl acetate was almost as effective as mineral oil in preventing loss in the weight of eggs and change in the pH of albumen. Carbon tetrachloride is equally as good a solvent for chlorinated rubber as ethyl acetate; in addition, it is non-inflammable. Carbon tetrachloride, however, is quite toxic to animals and should be used with caution, both in connection with a food product and in poorly ventilated places. Our search for another non-inflammable solvent possessing properties similar to those of ethyl acetate was fruitless.

B. *Use of Plasticizers in Plastic Egg-Coating Films.*

Many thermoplastic substances do not possess all the physical properties desired for a given practical use. To produce films with such properties as high permeability to water, low softening point, flexibility, and adhesiveness, synthetic resins are modified with chemicals known as plasticizers.

From a large number of compounds used as plasticizers, dibutyl phthalate and n-butyl stearate were selected for testing purposes. Both are compatible with each of the three plastics being tested, and have very little affinity for water. Because their volatilities are low and their boiling points high, each of these plasticizers can be expected to aid in producing films that will remain fairly stable during long storage periods. In addition, each is readily available in large quantities and is relatively inexpensive compared to most other plasticizers.

The use of either dibutyl phthalate or n-butyl stearate as a modifier of polyvinyl acetate yielded disappointing results. The addition of three per cent (by weight) of dibutyl phthalate to a 15 per cent solution of polyvinyl acetate (softening point—86.5°C.) in ethyl acetate produced a plastic film that was more permeable to water and carbon dioxide than an unmodified polyvinyl acetate film. Films of polyvinyl acetate modified with n-butyl stearate also allowed more rapid passage of water, as shown by the data (Table 2).

Polystyrene coatings containing either dibutyl phthalate or n-butyl stearate, on the other hand, retarded both the increase in alkalinity of the albumen and the rate of weight loss more than unmodified polystyrene films. During the 10-day storage period at 37.5°C., loss in weight from untreated eggs was 12 times greater than from eggs coated with a film composed of 10 parts polystyrene and four parts butyl stearate. At the end of the 10-day storage period, the pH of the albumen in eggs coated with this film was 7.7, which is the same as that of strictly fresh eggs. After storage, the appearance of the albumen and yolk of these coated eggs was not recognizably different from that of fresh eggs, whereas untreated eggs held for the same period under the same conditions were classed as inedible.

Chlorinated rubber films of the 125-centipoise type are very hard and soften at some temperature above 100°C.(212°F.). When dipped in boiling water, the shells of eggs coated with unmodified chlorinated rubber crack open, because the expanding gases in the egg are unable to penetrate through the highly impermeable film. In order to decrease this undesirable high softening point, plasticizers are usually added to chlorinated rubber. In addition, chlorinated rubber films containing either butyl stearate or dibutyl phthalate plasticizer were found to be highly effective in preventing eggs from losing weight and the albumen from becoming more alkaline (see data Table 2).

TABLE 2

Effect of Plasticized Plastic Coatings on Loss in Weight of Eggs and on pH of Albumen [1]

Plastic dissolved in ethyl acetate		Plasticizer		Relative loss in weight of eggs	Alkalinity of albumen
Type	Concentration by weight	Type	Concentration by weight		
	pct.		*pct.*	*pct.*	*pH*
Polyvinyl acetate	15.0	.....	.....	28	8.4
Polyvinyl acetate	15.0	n-Butyl stearate	3.0	33	8.4
Polyvinyl acetate	15.0	Dibutyl phthalate	3.0	37	8.8
Polystyrene	10.0	.....	.....	22	8.9
Polystyrene	10.0	Dibutyl phthalate	2.0	17	8.7
Polystyrene	10.0	Dibutyl phthalate	4.0	15	8.5
Polystyrene	10.0	n-Butyl stearate	4.0	8	7.7
Chlorinated rubber	15.0	.....	.....	23	9.0
Chlorinated rubber	15.0	n-Butyl stearate	4.2	14	8.0
Chlorinated rubber	15.0	Dibutyl phthalate	4.2	12	8.0
Chlorinated rubber	15.0	Dibutyl phthalate	2.1	9	8.0
Untreated eggs				100	9.4

[1] Eggs held for a 10-day period at 37.5°C.(99.5°F.) and about 35 per cent relative humidity.

C. *Other Modifications of Plastic Films.*

The rate at which carbon dioxide and water vapor escape from an egg is partly dependent upon the thickness of the plastic coating applied to the shell. A thicker film can be deposited by dipping the eggs in a more concentrated or more viscous plastic solution or by applying several coats from a dilute solution. Since application of thicker films from solution increases the drying time and the cost of materials used for treatment, consideration was given to further modifications of plastics with other substances that could possibly decrease the permeability of a thin film without changing its other physical properties.

In general, substances such as mineral oil, petroleum jelly, and paraffin wax are regarded as incompatible with the plastics discussed in this report. If added in sufficient quantities they tend to produce a cloudy film of low tear-strength and elasticity. When a plastic film containing petrolatum is allowed to dry, some petrolatum is squeezed out of the film and acts as a surface lubricant. If the film dries on the surface of an eggshell, some petroleum jelly is forced into the pores of the shell. Since it is a

good waterproofing agent, petrolatum should decrease the permeability of the eggshell. Experimental data confirming this supposition are presented (Table 3). Eggs dipped in plastic solutions containing 0.2 per cent of petrolatum lost less weight during storage than eggs coated with plastics containing none of this material. Physical properties, such as elasticity and tear-strength of the plastic film, were not noticeably influenced by small concentrations of petroleum jelly.

TABLE 3

Effect of Petrolatum and Microcrystalline Wax Upon Sealing Ability of Plasticized Plastics [1]

Plastic		Plasticizer		Petrolatum	Microcrystalline wax	Relative loss in weight	Alkalinity of albumen
Type [2]	Concentration [3]	Type [2]	Concentration [3]				
	pct.		*pct.*	*pct.*	*pct.*	*pct.*	*pH*
CR	15.0	BS	4.2	.....	.....	14	8.0
CR	15.0	BS	4.2	0.2	.....	10	8.0
CR	15.0	BS	4.2	0.2	0.1	6	7.9
CR	15.0	DP	4.2	.....	.....	12	8.0
CR	15.0	DP	4.2	.....	0.1	10	7.9
PS	10.0	DP	4.0	.....	.....	15	8.5
PS	10.0	DP	4.0	0.2	.....	13	8.3
PS [4]	10.0	BS	4.0	.....	.....	8	7.7
PS [4]	10.0	BS	4.0	0.2	0.1	4	7.9

[1] Eggs held for a 10-day period at 37.5°C. (99.5°F.) and about 35 per cent relative humidity. [2] Legend: CR = chlorinated rubber, PS = polystyrene, BS = n-butyl stearate, DP = dibutyl phthalate. [3] The concentration is expressed in terms of per cent by weight dissolved in ethyl acetate. [4] These mixtures contain 0.5 gram of cetyl pyridinium chloride per 1,000 ml. of solution.

Solutions of polystyrene and of chlorinated rubber appeared to be sensitive to the additions of any appreciable quantity of paraffin wax, ceresin, or microcrystalline wax. Paraffin wax formed a macrocrystalline precipitate in an ethyl acetate solution of plastic and plasticizer that was cooled to room temperature. Ceresin produced a fine microcrystalline dispersion in ethyl acetate and improved the impermeability of plastic films to water vapor, but it considerably increased the drying time and tackiness of the film. When used in small quantities, microcrystalline wax [5] decreased the passage of water vapor through plastic films (indicated by data Table 3). While this wax did not noticeably affect the other physical properties of chlorinated rubber films, it caused the polystyrene film to become soft and inelastic, so that more careful handling of the treated eggs was required in order to keep the film intact.

Inclusion of cetyl pyridinium chloride, a cationic detergent found to inhibit mold growth effectively, had no unfavorable effect on the impermeability of the plastic films (shown by data Table 3). This detergent is insoluble in ethyl acetate, but can be uniformly distributed in solution by adding five per cent (by volume) of ethyl alcohol to ethyl acetate. This small quantity of alcohol has no apparent adverse effect upon films of polystyrene and of chlorinated rubber.

When imperfectly dried plastic-coated eggs previously dipped in solutions or aqueous emulsions of plastics were stored in contact with each

[5] Sunwax 1290 supplied by The Sun Oil Company.

other, and the plastic allowed to harden, an attempt to separate the eggs usually resulted in a piece of one eggshell being torn off. To prevent surface tackiness of some slow-drying films, the coated eggs were rolled in finely powdered dry calcium carbonate. Calcium carbonate was incorporated into a polystyrene film to serve as a filler and to reduce stickness, but it reduced the tear-strength of the film noticeably.

D. *Preservation of Cracked Eggs by Plastic Coating.*

Losses in weight and changes in albumen pH of cracked eggs which were either coated with plastic or dipped in mineral oil are given (Table 4).

TABLE 4

Effectiveness of Plastic-Coating and Oil-Dipping in Preserving Cracked Eggs[1]

Treatment	Loss in weight per egg[2]		Relative loss in weight	Alkalinity of albumen
	gm.	*pct.*	*pct.*	*pH*
Plastic,[3] double coat	0.7	1.3	9	8.0
Plastic,[3] single coat	1.4	2.5	17	8.5
Mineral oil dip	7.8	13.0	89	9.3
Untreated, cracked eggs	8.3	14.7	100	9.4

[1] Eggs held for a 10-day period at 37.5°C.(99.5°F.) and about 35 per cent relative humidity. [2] A dozen eggs were used for each treatment. [3] The coating solution was composed of 10 per cent chlorinated rubber, four per cent n-butyl stearate, 0.4 per cent petrolatum, and 0.07 per cent microwax dissolved in ethyl acetate.

Oil treatment apparently had little effect upon losses of water and carbon dioxide from cracked eggs. On the other hand, a single coating of chlorinated rubber and n-butyl stearate sealed the crack as well as the rest of the shell surface and considerably retarded the escape of moisture and carbon dioxide. When double coats of this plastic were applied, cracked eggs were as well preserved as eggs with sound shells. Similar results were obtained with polyvinyl acetate.

E. *Relationship Between Carbon Dioxide and the Keeping Quality of Eggs.*

An egg, when cooled to room temperature immediately after being laid, is in equilibrium with an atmosphere containing about 10 per cent carbon dioxide, Sharp (1937). This gas gradually escapes until the interior of the egg is in equilibrium with air, which contains about 0.03 per cent carbon dioxide.

If the carbon dioxide in the fresh egg is retained by sealing agents, or if the egg is placed in an atmosphere with a high content of this gas, an increase in the alkalinity of the albumen is retarded and liquefaction of the dense albumen is largely prevented, Sharp (1929), Romanoff and Romanoff (1949). Movement of water from the albumen into the yolk also is inhibited, Smith (1932), and the density of the yolk therefore does not decrease to any appreciable extent. Since the specific gravity of the yolk remains as it is in the fresh egg—that is, very similar to that of the albumen, Romanoff (1940)—there is no marked tendency for the yolk to float to the top of the albumen and stick to the shell membranes. The

vitelline membrane, moreover, retains much of its original strength in the presence of a high level of carbon dioxide, Gane (1935).

The beneficial effects of retaining the carbon dioxide originally present in the egg were substantiated by our observations. When applied to a four- to eight-hour-old egg, any one of the coating agents which inhibited the increase in alkalinity of the albumen during subsequent storage, also prevented (1) liquefaction of the dense albumen, (2) increase in the yolk size, (3) decrease in the breaking-strength of the vitelline membrane. None of the eggs, sealed well the day they were laid, developed stuck yolks, even though the eggs were not turned during storage for 10 days at 37.5°C. and then for two months at room temperature (under conditions unfavorable for mold growth). Our tests indicate that infertile eggs, properly coated within a few hours after they are laid, can be expected to maintain their initial quality at high summer temperatures for at least 10 days.

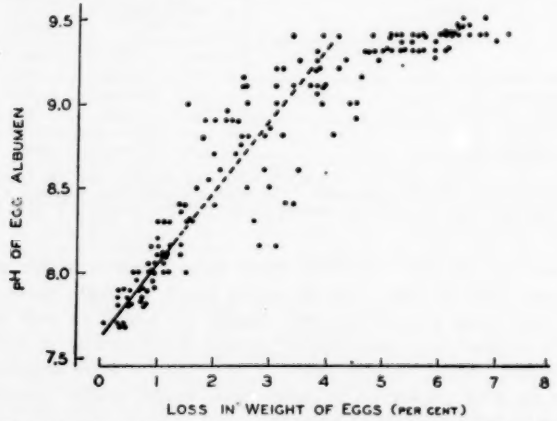

FIG. 1. Relationship between percentage loss in weight of eggs and pH of the albumen after a 10-day storage period at 37.5°C. (Each point represents the average value of six or more eggs.)

It has been reported that sealing the egg too soon after it is laid is inadvisable, because the amount of carbon dioxide initially present in some eggs may be sufficient to cause contraction of the mucin fibers and consequent cloudiness of the albumen. In our tests, however, cloudy albumen developed in only a few of the more than 1,000 eggs sealed with plastics or mineral oil four to eight hours after being laid. When newly laid eggs were stored at room temperature for 24 hours before being coated, enough carbon dioxide was lost to change the pH of the albumen to 8.3. Liquefaction of the albumen and swelling of the yolk then took place, in spite of the fact that further loss of carbon dioxide was prevented.

F. *Relationship Between Loss in Weight of Eggs and Alkalinity of Albumen.*

Weight lost by eggs during storage is mainly due to the escape of water. That this loss parallels the increase in the alkalinity of the albu-

men is shown (Fig. 1). During a 10-day storage period at 37.5°C., a decrease of five per cent or more of the eggs' weight was accompanied by a rise in the pH of the albumen to 9.4 or 9.5. For a given loss in weight between two and four per cent, significant variability in the pH of the albumen was noted. It is possible that these deviations may be due to a differential permeability, among the sealing agents, to moisture and carbon dioxide. On the other hand, when the weight loss of fresh eggs was less than one per cent, the pH of the albumen did not exceed 8.0. This indicates that the sealing agents which are highly impermeable to moisture can be expected to be very impervious to carbon dioxide, as well. The relative effectiveness of the superior coating agents in preserving eggs can be estimated, therefore, by simple weight measurements.

An approximate index of the preservative value of a coating method is provided by comparing the percentage loss in weight from an egg, sealed when fresh, with that from an untreated egg held in the same environment. Under our high temperature test conditions, a plastic-coated fresh egg which loses one-tenth as much weight as an untreated egg can be expected to maintain its original quality about 10 times as long as an untreated egg. If this approximation is applicable to other storage conditions, normal infertile eggs, coated when newly laid, can be expected to have nearly the same quality after a 10-month storage period as untreated eggs held for a one-month period under the same conditions (which must be non-conducive to microbiological growth). Further tests are necessary, however, to determine whether or not the stability of plastic films containing various plasticizers is affected during storage for long periods at low temperatures.

CONCLUSIONS

Newly laid eggs coated with films of chlorinated rubber, polystyrene, or polyvinyl acetate lose substantially less carbon dioxide and water during storage than untreated eggs. Ability of chlorinated rubber and polystyrene to preserve fresh eggs is improved by inclusion of either dibutyl phthalate or n-butyl stearate in the plastic solutions. The preservative value of these plasticized resins is somewhat increased by small amounts of petrolatum and microcrystalline wax. Inclusion of cetyl pyridinium chloride, an effective inhibitor of mold growth, does not decrease the sealing ability of plastic coatings.

Cracked eggs are as effectively sealed by plastic films as eggs with sound shells. Dipping cracked eggs in mineral oil does not decrease their perishability.

The change in appearance of the yolk and albumen in eggs coated when less than eight hours old is closely related to the loss of carbon dioxide and water. For best preservation the plastic coating of eggs should be carried out at the place of production, preferably the same day the eggs are laid. Plastic films, which are non-toxic, tasteless, and odorless, can be formed on eggs by a simple dipping or spraying method, without the use of elaborate equipment. Cost of the plastic materials and modifiers is not prohibitive for practical use.

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TRIPHENYL-TETRAZOLIUM CHLORIDE AS A VITAL STAIN FOR DRIED BAKERS' YEAST[1]

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In dried bakers' yeast the proportion of viable cells is of great importance. From the positive standpoint, the dried yeast is to be used in baking; hence there must be a sufficient number of viable cells to produce a satisfactory loaf of bread. On the negative side, where an excessive number of dead cells exist they have a deleterious effect upon the quality of the finished bread. It has been shown, Anonymous (1941), that while dried yeast was equal to compressed yeast as a gas producer, the dead cells contained a material which had a degrading action upon bread dough. Davis and Frenkel (1947) reported that when debittered dried yeast was added to dough for increased nutritive value, it produced a marked dough softening.

There are many methods in current use for determining the viability of yeast. The plating method requires at least three days, and according to Mills (1941) does not give an accurate estimate of the number of viable cells. He preferred the vital stain techniques, and gave directions for their use. However, even with careful control the authors have found it difficult to obtain reproducible results using methylene blue and erythrosin as vital stains.

The "punch" test, as recommended by the Quartermaster Corps of the United States Army (1944), consists of mixing a standard dough and measuring the length of time required for the dough to rise to a stated volume twice. A Chidlow dough expansion jar is used, and the gas is "punched" from the dough between the two rises.

The American Association of Cereal Chemists (1947) recommends testing yeast of unknown viability by incorporating it into a dough and baking bread. There are many instances of the use of this technique. Eisenberg (1941) has used the AACC procedure to produce the standard loaf of bread.

To test the viability of bakers' yeast, it may be mixed with flour and water and placed within a closed container. Both volume and pressure of gas produced may be used as indices of cell viability. Glabe (1942) described a manometric method. Eva, Geddes, and Frisell (1937) attempted to correlate results obtained with various types of gas production measuring devices. Morse (1948) described a method for testing the viability of yeast using two-inch-pipe fermenting chambers connected to manometers. Good correlation between the instrument described and the "punch" and baking tests was observed.

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Schaefer (1947) reported a method for measuring the activity of yeast on the basis of weight loss from inoculated media due to carbon dioxide production by yeast fermentation. He suggested using this procedure as a standard method.

Tetrazolium salts were first synthesized by Pechman and Runge (1894), but received little attention at that time. Renewed interest was shown when Bamberger and Billeter (1931) were able to prepare formazan, the reduced form of tetrazolium. Kuhn and Jerchel (1941a) were later able to oxidize formazan with the aid of lead tetraacetate. Mattson, Jensen, and Dutcher (1948) have recently published an improved method for the preparation of formazan. Kuhn and Jerchel (1941b) showed that tetrazolium salts, which they called "invert soaps" were reduced by bacteria, sprouting seeds, and fermenting yeasts. Lakon (1942) was able to predict the germinability of seeds by soaking them in an aqueous tetrazolium solution and later counting the red seeds. The viable seeds reduce the colorless tetrazolium to carmine-colored formazan. Porter, Durrell, and Romm (1947) confirmed the work of Lakon. Mattson, Jensen, and Dutcher (1947) found that tetrazolium salts were reduced by a variety of tissues, including bull sperm and egg blastoderm. It has been reported, Anonymous (1947), that formazan is not diffusible but is deposited in the tissues where it develops. The equation for the reaction follows:

Tetrazolium chloride (colorless) — Formazan (carmine)

In view of the peculiar property of being reduced to a carmine color by live tissue, it seemed possible that tetrazolium salts might be applied to the determination of the viability of bakers' yeast.

PRELIMINARY OBSERVATIONS

Two approaches to the problem were employed: 1) triphenyl-tetrazolium chloride was used in the customary stain techniques, and results observed microscopically; and 2) the dye was mixed with suspensions of yeast and the color development recorded, both visually and with an appropriate laboratory instrument.

MICROSCOPIC

Ten mg. of dried bakers' yeast of known low viability, according to pressuremeter analysis, were mixed with 10 ml. of one per cent aqueous solution of triphenyl-tetrazolium chloride. The mixture was swirled until the yeast was in complete suspension, then incubated at 30°C.(86°F.) for 20 minutes. Upon microscopic examination no differentiation could be detected between viable and non-viable cells. All cells appeared to have reduced the dye to a red color of equal intensity. Staining was largely peripheral, but occasional cells showed both central and peripheral staining. Long bacterial rods which appeared as contaminants took the

stain in much the same manner as the yeast, in that they showed a bright-red peripheral stain.

It was thought that cells in an active state of fermentation might reduce the dye more rapidly, and thus provide a means of differentiation. The above experiment was repeated, except that sucrose was added to the dye solution so that it contained three, two, and one per cent sucrose, respectively. Though color gradations were apparent, no microscopic differentiation could be observed. Further work is planned in which lower temperatures and reduced dye concentrations will be used, with the thought that differentiation may be more readily observed.

MACROSCOPIC

A series of experiments was undertaken to determine the factors which affect the reduction of triphenyl-tetrazolium chloride by suspensions of rehydrated dried bakers' yeast. In working with yeast in an active state of fermentation, it was observed that when sucrose was added to a yeast-dye mixture to produce fermentation, dye reduction took place more rapidly than it did in a control without added sucrose. This effect was considered to be due either to the altered physiological condition of the yeast during fermentation, or to the presence of reducing sugars brought about by the action of yeast enzymes upon sucrose.

In identical experiments where the only variable was yeast quantity, those suspensions containing larger amounts of yeast brought about a more rapid reduction of the dye.

Experiments in which different incubation temperatures were used for the yeast-dye suspensions, showed that higher temperatures brought about a more rapid reduction of the dye. This is in agreement with Mattson, Jensen, and Dutcher (1947) who established the reduction as an enzyme function.

That the amount of tetrazolium salt has an effect upon the reaction was shown when identical suspensions of yeast were mixed with graduated amounts of the dye. Upon incubation, color gradations were apparent which paralleled the amount of dye used.

To determine whether or not the color development was a function of yeast viability, mixtures of live and dead cells were prepared and tested. Dead cells were prepared by exposing yeast suspensions to a boiling water bath for 10 minutes. The suspensions were prepared by making three gm. of dried yeast up to 100 ml. with distilled water. Suspensions which contained more than 50 per cent of the cells as live cells were difficult to differentiate visually, using the dye test.

The experiments were repeated and the developed color examined with the aid of a Photovolt Reflection Meter, Model 610. Preliminary tests were conducted to determine the best filter to use for making these determinations. For standardizing the instrument, color standards were secured from the Munsell Color Corporation of Baltimore, Maryland. All color designations are those of the manufacturer. Color standards used to standardize the instrument for use with the suppressed zero method are shown. Of the different combinations of filter and color standards tried, that using the green filter and 2.5R-4/20 as zero, and 5R-5/12 as 10 gave the most widely

separated readings. Data using this combination are shown (Table 1). In conducting the test with the reflection meter it was found that the yeast suspension required shaking to insure complete suspension immediately before making a reading. Since the color developed within the yeast cells, the readings were affected if they were allowed to settle to the bottom of the test cuvette. Further, the nature of the cuvette containing the test material and its method of use were found to have an influence on the readings. In all determinations an oblong, optically-flat cuvette, with a capacity of 30 ml., was used. It was originally designed for use with the Coleman Universal Spectrophotometer, and is designated as Part 7103-RCK by the manufacturer. In use, the search unit of the reflection meter was inverted, and the cuvette placed upon the search unit so that the light source was completely covered by the bottom of the cuvette.

TABLE 1

Reflection Meter Readings[1] From Yeast Suspensions[2] Mixed With Triphenyl-Tetrazolium Chloride

Viable yeast	Sample number					
	1	2	3	4	5	6
pct.						
100	54	45	54	49	45	44
80	57	47	56	50	46	49
50	62	65	61	53	52	50
20	85	94	78	71	67	65
0	over 100	over 100	over 100	over 100	over 100	over 100

[1] Filter: green. Color standards: 0—2.5R-4/10, 10—5R-5/12. Incubation: 30 minutes at 40°C. (104°F.). [2] Three gm. dried bakers' yeast made up to 100 ml.

In hope that a greater differential could be obtained from instrument readings on the yeast viabilities above 50 per cent, attempts were next made to extract the developed color and measure it with a Coleman Universal Spectrophotometer, Model 11.

The first methods of color extraction attempted were physical in nature. Alternate freezing and thawing, autoclaving for 15 minutes at 121°C. (248°F.), and autolysis according to Neuberg and Roberts (1946), failed to release all the color; much of it remained fixed in the cellular debris.

Solvents were tried next, including ethyl alcohol, petroleum ether, methyl alcohol, glacial acetic acid, dioxane 1, 4, benzene, toluene, isobutyl alcohol, isoamyl alcohol, acetone, ethyl ether, ethyl acetate, and chloroform. Ethyl alcohol, dioxane 1, 4, and acetone partially extracted some of the color. Dioxane was discarded because the extracted color bleached rapidly upon standing, and acetone was selected over ethyl alcohol because it extracted more of the color. Further attempts to extract all the color included repeated washings with acetone, and different acetone and yeast ratios of mixture. In none of these experiments was the color completely removed. The color extracted by acetone was examined for spectral transmission characteristics. A maximum of absorption was observed in the region of 475 mμ., as shown (Fig. 1), which is in close agreement with the data of Kuhn and Jerchel (1941b).

Methods of destroying the cells with strong acids and alkalis, for the purpose of releasing the color, were also tried. Sulfuric, hydrochloric, and nitric acids, both concentrated and diluted 1:1, failed to release all color from the cells. Extraction with 50 per cent potassium hydroxide freed the color from the cells, but a milky suspension resulted. Preliminary attempts to clarify the suspension by neutralizing with sulfuric acid and salting out with either ammonium chloride or phosphotungstic acid were not successful. This approach shows promise, in that in all previous

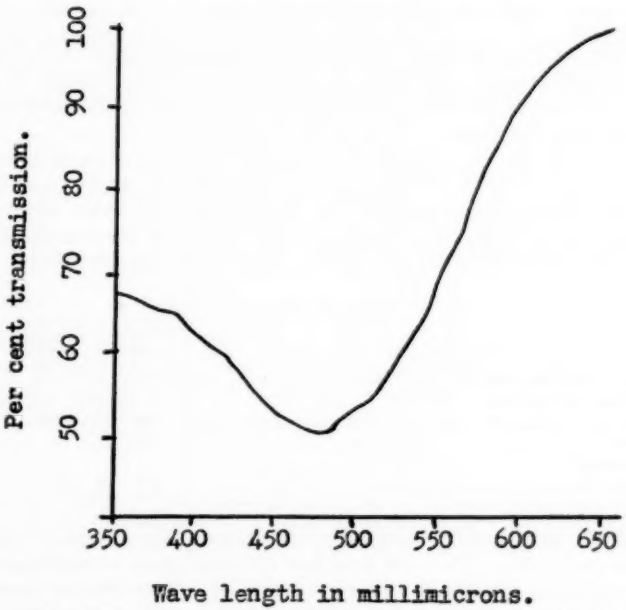

FIG. 1. Transmission characteristics of acetone-extracted color developed in yeast by triphenyl-tetrazolium chloride.

attempts at extraction colored cellular debris could be separated by means of a fritted glass funnel. However, extraction with 50 per cent potassium hydroxide left no residue. Since no residue was left, and microscopic examination failed to show intact cells, it appeared that the yeast cells had been destroyed and the milky suspension contained the cellular contents which had been released. Further work is planned to attempt to remove the suspended material, or to extract the color from the suspension.

While color extraction with acetone was not complete, regardless of the ratio of the dyed cells and solvent, it tended to be proportional. The more color the cells developed, the more color was extracted by acetone. Yeast suspensions of graduated viability were mixed with triphenyl-tetrazolium chloride solution, incubated, and then extracted with acetone. After separation on a fritted glass funnel the color was measured on a

Coleman Universal Spectrophotometer, Model 11. Typical data are shown below:

Yeast viability	Light transmission at 475 mμ.
pct.	*pct.*
100 alive	74.0
80	75.5
50	80.5
20	83.0
0	85.0

It will be observed that while the same general pattern is followed in the spectrophotometer results as in the reflection meter results, there is a larger differential between reflection meter readings than between spectrophotometer readings.

As a check on the acetone extraction method three lots of yeast were examined: one of high viability, one of low viability, and one of medium viability. They were examined by the acetone extraction method, the "punch" method, and the pressuremeter method in accordance with the procedure described by Morse (1948). Results are shown below:

	High viability yeast	Medium viability yeast	Low viability yeast
Per cent light transmission at 475 mμ	64.5	74.2	78.5
Punch time in minutes:			
First	135	175	360
Second	85	95	.....
Pressuremeter:			
Reading mm. mercury for two-hour test period	487	332	115

The above data show good correlation between the "punch," dye, and pressuremeter tests. Previous tests had indicated good correlation between results secured with the spectrophotometer and the reflection meter.

The following method is suggested as a test for yeast of unknown viability:

1) Prepare a suspension of dried yeast to be examined for viability by making three gm. up to 100 ml., using distilled water, 40°C.(104°F.). For compressed yeast use a 10-gm. sample. Prepare a similar suspension using yeast of know viability of identical moisture or corrected moisture content.

2) Mix 49 ml. of the above suspension with one ml. of a one per cent aqueous solution of triphenyl-tetrazolium chloride. Incubate the thoroughly mixed samples for 30 minutes at 40°C.(104°F.).

3) Using a reflection meter, read the developed color by the suppressed zero method. For establishing the proper range, use Munsell Color 2.5R-4/10 to adjust the instrument to zero, and 5R-5/12 to adjust it to 10. Before filling the test solution into a 30-ml. optically-flat cuvette, such as Wilkens-Anderson Company, 7103-RCK, mix suspension thoroughly. Read immediately after pouring. For making a reading, invert the search unit of the instrument and completely cover the light source with the test sample.

4) Compare the readings of the yeast of known and unknown viability. If the unknown has a lower reading, it has higher viability.

SUMMARY AND CONCLUSIONS

The use of triphenyl-tetrazolium chloride as a microscopic vital stain shows that live and dead yeast cells reduce the dye to a red color of equal intensity. The resulting stain is largely peripheral in nature, with occasional center staining. Unidentified long bacterial rods reduced the dye to a bright-red color, indicating the possibility of using triphenyl-tetrazolium chloride as a bacteriological stain.

Of the macroscopic methods employed, that using the reflection meter to measure difference in color development as related to yeast viability, gave the best results. Visual examination of the developed color was the least satisfactory; yeast in the range of 50 to 100 per cent viable could not be clearly differentiated on the basis of color developed. Extraction of the developed color with acetone and measuring percentage light transmission of the extract with the spectrophotometer was more satisfactory than visual examination, but less satisfactory than reflection meter determinations. Good correlation was obtained between the "punch" test, the pressuremeter test, the spectrophotometer, and reflection meter methods. Examination of the color developed in yeast by the action of triphenyl-tetrazolium chloride, with the use of the reflection meter, is a satisfactory means of determining yeast viability, especially in the lower ranges of viability.

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VITAMIN AND AMINO ACID CONTENT OF PROCESSED CHICKEN MEAT PRODUCTS[1]

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Developments in the methods of food preservation applied to poultry have brought about a wider distribution and greater diversity of chicken meat products available for consumption. Chicken meat is recognized not only as a valuable source of food protein but also as an important source of the B vitamins. While numerous studies have been made on the vitamin content of chicken meat, few data are available at present regarding the effects of different processing methods on retention of the vitamins in the common processed products. Similarly, little information is available in regard to the effect of processing on the amino acid composition of the protein. The dearth of such information has heretofore, to a large extent, been attributable to the absence of precise methods for measurement of these compounds. Recent application of microbiological methods to the assay of amino acids, however, now makes possible the detection of relatively small differences in the amino acid composition of proteins and foods.

Canning, freezing, and curing and smoking are the most commonly used methods for preservation of poultry products. In the present investigation, a study was made of the effects of these methods of processing on the retention of certain of the B vitamins, and the so-called "indispensable" amino acids.

VITAMIN CONTENT OF CHICKEN MEAT AND STABILITY OF VITAMINS IN THE PROCESSING OF MEATS

Meats are generally good sources of the B vitamins, and extensive studies have been made on the distribution of these vitamins in the various common meats. Vitamins of other than the B group have been found only in very small amounts, however, with significant values evident only in liver, and perhaps some other visceral organs.

Booher and Marsh (1941) could detect no vitamin A in chicken meat in studies by the rat growth method. Guilbert and Hart (1934) found 162 to 250 I. U. of vitamin A per 100 gm. of turkey liver, and 625 to 675 I. U. of this vitamin per 100 gm. of chicken liver.

The vitamin D content of meats is likewise negligible. Munsell (1940) reported 50 I. U. of this vitamin per 100 gm. of chicken liver, but few references could be found in the literature on determinations of this vitamin in meats.

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The ascorbic acid content of chicken muscle, as reported by Bessey and King (1933) was four mg. per 100 gm. of meat, and 28 mg. per 100 gm. of chicken liver.

Studies on the thiamine content of chicken meat by various methods, Booher and Hartzler (1939), Mickelsen *et al.* (1939), Leong (1939), and Cheldelin and Williams (1942), revealed values for thiamine in the various portions of chicken meat ranging from 30 μg. up to 270 μg. per 100 gm. Dark meat was found to show consistently higher values. Rice *et al.* (1946), in a very careful study of differences in vitamin contents of 13 different muscles of chicken, found values ranging from 0.4 to 1.3 μg. of thiamine per gm. of meat, with dark meat containing two to three times as much thiamine as light muscle.

Pyke (1939) found 51 μg. of thiamine per 100 gm. of roasted dark chicken meat in determinations made by chemical methods, and a value of 120 μg. per 100 gm. when the rat growth method was used. Baker and Wright (1935) also reported a value of 120 μg. per 100 gm. for roasted chicken meat.

Studies on the riboflavin content of chicken meat have indicated a range of values from 50 to 350 μg. per 100 gm. Mickelsen *et al.* (1940), using microbiological methods, found values for riboflavin in light and dark chicken meats of 0.8 and 2.6 μg. per gm., respectively. Similar values were reported by Schörmuller (1939) and Cheldelin and Williams (1942). Hudson (1940) found the values to vary, depending on whether the chickens were fed diets high or low in riboflavin. Rice *et al.* (1946) found a range of values from 0.5 to 3.5 μg. per gm. in various portions of chicken meat, and observed that, similar to the findings in studies of thiamine, dark muscles contained two to three times as much riboflavin as light muscles.

Although no data were found on the effect of processing on the riboflavin content of chicken meat, factors affecting the stability of this vitamin in other types of meat are of interest. McIntire and co-workers (1943) and Schweigert and co-workers (1943) reported 85 per cent retention of riboflavin after roasting, broiling, and braising of meat. Retention of riboflavin after cooking of lamb and veal ranged from 87 to 101 per cent. Storage, curing, and cooking were likewise found to cause only small losses. Rice and Robinson (1944) reported 90 to 100 per cent retention of riboflavin in canned pork and beef, with no additional loss during storage for seven months.

Determinations of nicotinic acid in chicken meat by McIntire and coworkers (1943) revealed values of 7.3 mg. per 100 gm. of light meat, and 6.1 mg. per 100 gm. of dark meat. Livers were found to contain 14.1 to 17.8 mg. per gm. Cheldelin and Williams (1942) reported analyses of 170 μg. per gm. of dark meat, and 360 μg. per gm. of light meat. Rice *et al.* (1946), Waisman and Elvehjem (1941), Kringstad and Thorensen (1940), and Giri and Naganna (1941) likewise reported similar values for nicotinic acid in chicken meat, all analyses revealing a slightly higher percentage of the vitamin in light than in dark meat.

With respect to retention of nicotinic acid in chicken meat during preparation, Dann and Handler (1941, 1942) reported losses ranging from

31 to 64 per cent during frying, 26 to 37 per cent during roasting, and 34 to 46 per cent during steaming. The stability of nicotinic acid in other meats has been more widely investigated. McIntire, Schweigert, and Elvehjem (1943) reported 65 to 100 per cent retention of nicotinic acid in meat during cooking, with most values above 90 per cent. Dann and Handler (1941, 1942) found that losses due to cooking of meats ranged from 33 to 50 per cent, on the dry-weight basis. In canned meats, Rice and Robinson (1944) found 90 to 100 per cent retention.

Waisman *et al.* (1942) reported the pantothenic acid content of chicken meat as nine μg. per gm. of light meat, and 21 μg. per gm. of dark meat. Cheldelin and Williams (1942) found 6.2 and 5.3 μg. per gm. of dark and light meat, respectively. Rice and co-workers (1946) found ranges of from 11 to 40 μg. per gm., with values again showing proportions of from two to three times more of the vitamin in dark than in light meat.

Losses of pantothenic acid during cooking and processing of meats were found by Waisman *et al.* (1942) to range from very slight amounts up to 66 per cent. Rice and Robinson (1944) reported average retention values of 70 to 80 per cent of the vitamin during the canning of meats, with no further change after seven months of storage.

Cheldelin and Williams (1942) reported values for both biotin and folic acid in chicken meat. Biotin was found to be present in amounts of 0.098 and 0.054 μg. per gm. of dark and light meat, respectively, and folic acid was determined in an amount of 1.2 μg. per gm. of dark meat, and 1.5 μg. per gm. of light meat.

Investigations by Schweigert *et al.* (1943, 1946) on the stability of these vitamins during cooking revealed that biotin was retained in an amount of 80 per cent, but retention of folic acid varied from 10 to 65 per cent.

EFFECT OF HEAT ON NUTRITIVE VALUE OF PROTEINS

Cooking or processing of proteins has been found to result in a loss of nutritive value of the protein. Investigations have revealed that these losses are in most instances very small, and whether the reduction is owing to destruction of certain amino acids, decreases in the digestibility of the protein, or other factors, has not yet been clearly established.

Richet (1925) fed dogs with raw, cooked, and overcooked meat, and found that metabolism of the protein of raw meat is more complete than with cooked meat. Morgan and King (1926, 1931) found that toasting decreased the growth-supporting value of cereal proteins. When cereals were cooked with water, however, a slight increase in nutritive value of protein for growth was observed.

Okuda and Yamafuji (1933) processed the protein of hen, whale, and fish at 140°C.(284°F.) for one hour in sealed containers, and found that about 20 per cent of the protein nitrogen had been hydrolyzed, and that the contents of arginine, histidine, and lysine were decreased. Values for tryptophane and tryosine changed only slightly, and the value for proline remained the same.

Morgan and Kern (1934) found that raw beef had a greater biological value than cooked beef, the difference being attributed to some injury to the protein during cooking. Stewart and co-workers (1943) found that

precooking, household preparation, and drum drying do not affect the protein quality of oats, but that explosion techniques used in the preparation of breakfast foods do cause considerable damage to oat protein.

EXPERIMENTAL PROCEDURE

Preparation of Samples: White Rock chickens (fowls) obtained from the Department of Poultry Husbandry of the University of Massachusetts were used for experimental studies. The birds had been raised under similar conditions, and had been fed a complete, practical diet to assure, insofar as possible, similar composition of the muscle flesh. The birds were starved for 24 hours, then killed by piercing the brain and severing the jugular vein to permit complete bleeding. After bleeding had ceased, the birds were defeathered with a mechanical picker, and immediately dressed, washed, and dried with paper towels.

Seven different samples were prepared, each consisting of four birds selected at random. One sample was used for the determination of amino acids and vitamins in the fresh muscle tissues and visceral organs. A second was prepared for an investigation of the nutritive value of chicken meat protein [results of which have been reported previously by the authors (1948)]; and a third for a study of the effect of cooking. The remaining four samples were processed in such a manner as to represent commercial packs of chicken meat in glass jars and tin cans, as well as smoked and cured, and frozen meats.

For preparation of the fresh samples, the chickens were boned, and the meat separated into two parts, one consisting of light meat from the breast and wing muscle flesh, the other of dark meat from the muscles of the legs and thighs. Livers and gizzards were also saved for analysis. All visible fat was removed from the muscles, and the different portions passed several times through a grinder with holes 0.5 mm. in diameter. After thorough mixing to assure homogeneity, samples were drawn from each portion for vitamin determinations, and the remainder of each portion packed in hermetically-sealed glass jars and frozen (in a manner previously described by the authors), for amino acid analyses.

Studies on the effects of cooking were carried out with similarly prepared samples. After dressing, the chicken carcasses were placed in a kettle and steamed for 40 minutes. Livers and gizzards were cooked as separate samples, with approximately one quart of water per 600 grams of each organ. This amount of water was just sufficient to cover the meat. At the end of the cooking period, the chickens were boned and the meat separated, as before, into light and dark portions. All portions were then thoroughly ground and mixed until homogeneous. These samples were packed in hermetically-sealed glass jars, frozen, and stored at —18°C. (—0.4°F.) until used.

For canning studies, the chickens were divided into two sets. In one set, the birds were steamed at 121°C.(248°F.) for 20 minutes, then boned. The meat was then divided into light and dark portions which were canned separately. The steamed chicken pieces were packed in No. 2 (307 x 409) "C" enamel cans, covered with the broth obtained during steaming, and sealed. Since the filling temperature was 54°C.(129°F.), the cans were

processed for 70 minutes under 15 pounds' steam pressure, 121°C. After processing, the cans were opened, the whole contents ground and mixed in the same manner as the previous samples, transferred to glass jars, hermetically sealed, and stored at —18°C. until portions were drawn for analysis.

In the second group, the meat was canned as follows: the carcasses were cut into pieces consisting of breast, thighs, legs, and wings, placed in trays, and roasted in a moderate oven, 176°C.(349°F.) for 30 minutes. After roasting, the pieces were boned out, put into pint glass jars, covered with the hot drippings, and the jars sealed and processed under 15 pounds' pressure (121°C.) for 85 minutes. When processing and cooling were complete, the jars were opened, and the whole contents ground and mixed, as with previous samples. These samples were likewise stored in glass jars at —18°C. until tests were made.

TABLE 1

Thiamine Content of Chicken Meat and Chicken Meat Products and Its Retention During Processing

Sample	Light meat		Dark meat		Livers' content	Gizzards' content
	Content	Retention	Content	Retention		
	μg./gm.	pct.	μg./gm.	pct.	μg./gm.	μg./gm.
Fresh	0.97	.....	1.76	.....	2.70	0.97
Frozen	0.85	87.60	1.02	58.00	.....	.....
Precooked frozen	0.55	56.70	0.56	31.60	.....	.....
Canned (tin cans)	0.32	33.00	0.41	23.30	.....	.....
Canned (glass jars)	0.44	45.40	0.41	23.30	.....	.....
Cured, smoked, and canned	0.29	30.00	0.29	16.50	.....	.....

For cured, smoked, and canned samples, the washed and dried carcasses were placed in a curing solution consisting of two lb. salt, 0.5 lb. sugar, one oz. sodium nitrate, and 0.2 oz. sodium nitrite, made up with water to five gallons. The average dressed weight of the birds was three pounds, and allowing a curing time of one and one-half days per pound of dressed weight, a total of four and one-half days was employed in the curing process. At the end of this period, the carcasses were removed from the curing solution and drained by hanging in a refrigerator room, at a temperature of 4.5°C.(40°F.), for 12 hours. After draining the birds were removed to a smoke house, and smoked for eight hours. The temperature of the smoke house during the smoking process ranged between 49 to 54°C.(120 to 129°F.). When smoking was completed, the carcasses were boned out, the pieces packed into No. 2 short (307 x 400) "C" enamel cans, covered with boiling broth, and the cans sealed and processed at 121°C. for 75 minutes. After processing, the cans were opened and the whole contents thoroughly ground, mixed, and packed and stored in the usual manner.

Since freezing has been found to have no detectable effect on the amino acid content of tissues, vitamin determinations only were made on frozen samples. Studies were made with both fresh and precooked samples, prepared and packed as previously described, and stored at —18°C. for eight months.

RETENTION OF THIAMINE, RIBOFLAVIN, AND NICOTINIC ACID IN PROCESSED CHICKEN MEAT PRODUCTS

Analyses for thiamine, riboflavin, and nicotinic acid were carried out with both fresh and processed samples in order to determine the retention of these vitamins during the various processes. Determinations were made as soon as possible after treatment.

The thiochrome method of Hennesy (1942) was employed for determination of thiamine. Results of analyses for the vitamin in fresh light meat, dark meat, livers, and gizzards, and the percentage retention of the vitamin in the various processed products are shown (Table 1). Values for thiamine in the different portions are in agreement with those reported in the literature, and reveal that dark muscle contains a higher percentage of the vitamin than light muscle meat.

Retention values show that significant amounts of thiamine are destroyed during processing of chicken meat, the extent of losses depending on both the time and temperature required for the particular process. This relationship is indicated by the fact that canned, and cured, smoked and canned meats showed the greatest losses. Such losses can be explained by the instability of thiamine at high temperatures in media with pH values close to neutrality. The pH values of these products ranged from 6.0 to 6.2.

TABLE 2

Riboflavin Content of Chicken Meat and Chicken Meat Products and Its Retention During Processing

Sample	Light meat		Dark meat		Gizzards' content
	Content	Retention	Content	Retention	
	μg./gm.	*pct.*	*μg./gm.*	*pct.*	*μg./gm.*
Fresh	0.90	.....	1.80	.....	1.80
Frozen	0.88	97.0	1.60	89.0	.....
Precooked frozen	1.10	122.0	1.70	94.5	.....
Canned (tin cans)	0.90	100.0	2.00	111.0	.....
Canned (glass jars)	1.00	110.0	1.90	105.0	.....
Cured, smoked, and canned	1.06	118.0	2.10	116.0	.....

Riboflavin was determined in the various samples by the microbiological method of Snell and Strong (1939) using *Lactobacillus casei epsilon* as a test organism. Results of analyses are shown (Table 2), along with values for retention of the vitamin in the various processed samples. Values for riboflavin in the various portions of the fresh meat correspond with values found in the literature, and reveal that along with thiamine, riboflavin likewise is found in higher percentage in dark muscle than in light muscle meat.

Data obtained from analyses of the processed samples indicate retention values of 100 per cent or higher. Similar retention values after cooking and processing have been reported previously, and have been attributed to liberation during processing of riboflavin occurring in fresh meat as part of complex molecules.

Nicotinic acid was determined according to the microbiological method of Snell and Wright (1941) using *Lactobacillus arabinosus 17-5* as the

test organism. Values for the vitamin in fresh chicken meat portions, and retention values for the different processed products, are shown (Table 3). Results of determinations on fresh samples are in close agreement with values found in the literature, and confirm the findings of previous workers that nicotinic acid appears in greater quantity in light muscle than in dark muscle meat.

TABLE 3

Nicotinic Acid Content of Chicken Meat and Chicken Meat Products and Its Retention During Processing

Sample	Light meat		Dark meat		Livers' content	Gizzards' content
	Content	Retention	Content	Retention		
	μg./gm.	*pct.*	*μg./gm.*	*pct.*	*μg./gm.*	*μg./gm.*
Fresh	97.0	.....	48.0	.....	97.0	48.0
Frozen	107.0	110.0	48.0	100.0	.....	.....
Precooked frozen	68.0	70.0	45.0	94.0	.....	.....
Canned (tin cans)	94.0	97.0	46.0	96.0	.....	.....
Canned (glass jars)	100.0	103.0	45.0	94.0	.....	.....
Cured, smoked, and canned	81.0	84.0	40.0	83.5	.....	.....

Retention of the vitamin in the different processed samples varied from 70 to 110 per cent. While no apparent losses resulted from freezing of the meats, apparent losses were observed with the precooked, frozen meats, amounting to as high as 30 per cent in the light meat portion. Significant losses were noted in samples of meat cured, smoked, and then canned, but apparently little loss resulted from the canning process itself.

Retention of Amino Acids During Cooking and Processing: For estimation of amino acids retained during processing, the same microbiological methods and general procedures previously reported by the authors were employed. Prior to determinations of the individual amino acids, the protein content of each sample was calculated from the percentage of nitrogen determined by the Kjeldahl method, using the accepted conversion factor of 6.25 (N x 6.25). Calculated protein contents of the various processed samples are given (Table 4). These values are higher than those found

TABLE 4

Protein Content of Cooked and Processed Chicken Meat [1]

	pct.
Light meat:	
Cooked (steamed)	33.65
Canned (tin cans)	31.10
Canned (glass jars)	30.55
Cured, smoked, and canned	27.30
Dark meat:	
Cooked (steamed)	29.40
Canned (tin cans)	28.30
Canned (glass jars)	28.85
Cured, smoked, and canned	27.30
Livers:	
Cooked (water)	24.80
Gizzards:	
Cooked (water)	27.35

[1] Calculated to 16 per cent nitrogen.

for fresh meat, owing to losses of water during cooking or processing. Since the moisture losses in the samples would result in higher values for the amino acids in the processed samples, if comparisons with fresh samples were made on a weight basis, all values for amino acids were calculated on the basis of protein content.

TABLE 5

Leucine and Isoleucine Contents of Chicken Meat Products and Their Retention During Cooking and Processing[1]

Sample	Leucine			Isoleucine		
	In sample	In protein	Retention	In sample	In protein	Retention
Light meat:	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*
Fresh	.....	6.95	.....	.....	5.32	.....
Cooked	2.15	6.40	92.0	1.96	5.83	109.0
Canned (tin cans)	2.22	7.15	102.0	1.91	6.14	115.0
Canned (glass jars)	2.12	6.95	100.0	1.68	5.50	103.0
Cured, smoked, and canned	1.88	6.90	99.5	1.26	4.62	86.5
Dark meat:						
Fresh	.....	7.17	.....	.....	5.70	.....
Cooked	2.02	6.90	96.0	1.65	5.62	98.5
Canned (tin cans)	2.03	7.18	100.0	1.65	5.84	102.0
Canned (glass jars)	2.05	7.10	99.0	1.41	4.90	86.0
Cured, smoked, and canned	1.91	7.00	97.5	1.31	4.78	84.0
Livers:						
Fresh	.....	8.15	.....	.....	5.55	.....
Cooked	1.60	6.45	79.0	1.04	4.20	76.0
Gizzards:						
Fresh	.....	6.00	.....	.....	4.40	.....
Cooked	1.63	5.96	99.5	1.43	5.24	118.0

[1] Values for the per cent in protein calculated to 16 per cent nitrogen.

Leucine and isoleucine contents of cooked and processed chicken meat, and percentage retention of these amino acids in the various products are reported (Table 5). Retention of leucine in the samples varied from 92 to 102 per cent, except with liver, in which retention after cooking was only 77 per cent. Low retention values were found for cooked light and dark meat, while for the same portions canned, losses were small. These differences may be accounted for by the fact that some of the amino acids in the cooked meat could be lost in the drippings during cooking, whereas when the meats were canned, the drippings were retained in the samples and no loss was encountered. Curing and smoking resulted in only relatively small losses. Results in general indicate good retention of leucine in cooking and processing of chicken meat.

Retention of isoleucine ranged from 84 to 115 per cent in the various samples, again except for liver, which showed only 76 per cent retention of the amino acid. For cooked and canned meats, losses were small. The low retention value for dark meat canned in glass jars does not correspond with the rest of the findings, but since it is, nevertheless, within the limits of error of analysis, it may be considered as an abnormal value. Corresponding losses occurred in both light and dark meat during curing and smoking, suggesting that some loss of isoleucine occurs during the curing

process or in the drippings produced by the heat of the smoking process.

Values for valine and phenylalanine in cooked and processed chicken meat, and percentage retention of these amino acids, are shown (Table 6). Retention values for valine ranged from 94 to 116 per cent, with all values lying above 100 per cent, except for cooked liver. No losses of valine in light and dark meat appeared to result from any of the processes studied. Cooking with water, however, results in some loss of valine from the livers.

TABLE 6

Valine and Phenylalanine Contents of Chicken Meat Products and Their Retention During Cooking and Processing[1]

Sample	Valine			Phenylalanine		
	In sample	In protein	Retention	In sample	In protein	Retention
Light meat:	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*
Fresh	.....	4.72	.....	.....	3.78	.....
Cooked	1.76	5.23	110.0	1.41	4.19	110.0
Canned (tin cans)	1.71	5.50	116.0	1.30	4.21	111.0
Canned (glass jars)	1.59	5.20	110.0	1.27	4.15	110.0
Cured, smoked, and canned	1.42	5.20	110.0	1.57	4.30	113.0
Dark meat:						
Fresh	.....	4.56	.....	.....	4.02	.....
Cooked	1.43	4.85	106.0	1.26	4.30	106.0
Canned (tin cans)	1.50	5.30	116.0	1.26	4.45	110.0
Canned (glass jars)	1.40	4.85	106.0	1.25	4.34	108.0
Cured, smoked, and canned	1.45	5.31	116.0	1.24	4.56	113.0
Livers:						
Fresh	.....	5.55	.....	.....	4.60	.....
Cooked	1.30	5.24	94.5	1.16	4.68	101.0
Gizzards:						
Fresh	.....	3.78	.....	.....	3.18	.....
Cooked	1.21	4.29	113.0	1.10	4.03	126.0

[1] Values for the per cent in protein calculated to 16 per cent nitrogen.

Retention values for phenylalanine ranged from 101 to 113 per cent. The value of 126 per cent retention in cooked gizzards is considered abnormal, despite the fact that the value given represents an average of results obtained from two separate assays of duplicate samples. Since retention values are high with all samples, no losses of phenylalanine are indicated for the processes studied.

Results of studies on tryptophane and threonine retention are presented (Table 7). Retention values ranging from 48 to 83 per cent of the original tryptophane content of the various portions indicate significant losses of this amino acid under all processing conditions. The value of 140 per cent retention in liver, representing an average of three duplicate determinations, is considered abnormal in the light of other results. Analysis of the data reveals that greater losses of tryptophane occurred during canning than were encountered in cooking. These losses may run as high as 50 per cent when chicken meat is processed at 121°C. for 70 minutes.

Results of threonine determinations reveal retention values ranging from 92 to 115 per cent. These values indicate no significant losses of threonine during cooking or processing, although small losses of the amino acid are apparent when livers are cooked with water.

TABLE 7

Tryptophane and Threonine Contents of Chicken Meat Products and Their Retention During Processing[1]

Sample	Tryptophane			Threonine		
	In sample	In protein	Retention	In sample	In protein	Retention
Light meat:	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*
Fresh	.....	1.20	.....	.....	4.25	.....
Cooked	0.24	0.71	58.0	1.37	4.07	96.0
Canned (tin cans)	0.18	0.58	48.5	1.29	4.51	98.0
Canned (glass jars)	0.20	0.66	55.0	1.52	5.00	115.0
Dark meat:						
Fresh	.....	0.92	.....	.....	4.35	.....
Cooked	0.21	0.72	78.5	1.45	4.94	113.0
Canned (tin cans)	0.17	0.60	65.0	1.30	4.60	105.0
Canned (glass jars)	0.22	0.76	82.5	1.30	4.51	104.0
Livers:						
Fresh	.....	0.74	.....	.....	5.10	.....
Cooked	0.26	1.05	140.0	1.17	4.72	92.5
Gizzards:						
Fresh	.....	0.76	.....	.....	4.35	.....
Cooked	0.18	0.66	87.0	1.30	4.76	105.0

[1] Values for the per cent in protein calculated to 16 per cent nitrogen.

Values for retention of histidine and arginine are shown (Table 8). Retention of histidine ranged from 62 to 93 per cent, revealing relatively small losses of the amino acid during steaming and processing. A value of 62 per cent retention in livers cooked in water indicates that a significant loss of histidine occurs under these conditions.

Values ranging from 91 to 108 per cent retention were found in determinations of arginine, again with the exception of livers, in which only 80

TABLE 8

Histidine and Arginine Contents of Chicken Meat Products and Their Retention During Cooking and Processing[1]

Sample	Histidine			Arginine		
	In sample	In protein	Retention	In sample	In protein	Retention
Light meat:	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*
Fresh	.....	3.69	.....	.....	5.92	.....
Cooked	1.09	3.24	88.0	2.14	6.40	108.0
Canned (tin cans)	1.01	3.27	89.0	1.80	5.80	98.0
Canned (glass jars)	0.96	3.16	86.0	1.70	5.60	94.5
Dark meat:						
Fresh	.....	2.88	.....	.....	6.10	.....
Cooked	0.76	2.62	91.5	1.79	6.10	100.0
Canned (tin cans)	0.75	2.68	93.0	1.57	5.55	91.0
Canned (glass jars)	0.71	2.46	86.0	1.60	5.55	91.0
Livers:						
Fresh	.....	3.80	.....	.....	7.05	.....
Cooked	0.58	2.06	62.0	1.40	5.64	80.0
Gizzards:						
Fresh	.....	2.48	.....	.....	5.55	105.0
Cooked	0.34	1.11	45.0	1.60	5.85	105.0

[1] Values for the per cent in protein calculated to 16 per cent nitrogen.

per cent of the original amount of the amino acid remained after cooking. Muscle portions showed retention of 100 per cent or higher after cooking, but after processing lower values were obtained, suggesting that while there is little or no loss of arginine during cooking, an apparent loss occurs during processing.

Retention values for lysine ranged from 84 to 108 per cent as shown (Table 9). Values are higher for cooked or processed light meat than for dark meat subjected to similar treatment, but analysis of all values indicates that losses of lysine are small.

TABLE 9

Lysine Content of Chicken Meat Products and Its Retention During Cooking and Processing[1]

Sample	Lysine		
	In sample	In protein	Retention
Light meat:	*pct.*	*pct.*	*pct.*
Fresh	.....	7.50	.....
Cooked	2.74	8.10	108.0
Canned (tin cans)	2.47	7.96	106.0
Canned (glass jars)	2.14	7.00	93.5
Dark meat:			
Fresh	.....	8.75	.....
Cooked	2.27	7.75	89.5
Canned (tin cans)	2.02	7.40	84.4
Canned (glass jars)	2.26	7.70	89.5
Livers:			
Fresh	.....	7.30	.....
Cooked	1.66	6.70	92.0
Gizzards:			
Fresh	.....	6.00	.....
Cooked	1.86	6.80	113.0

[1] Values for the per cent in protein calculated to 16 per cent nitrogen.

DISCUSSION

Evaluation of results of vitamin determinations in chicken meat and chicken products reveals close agreement between values found in the present investigation and values found in the literature. Among the vitamins for which determinations were made, thiamine showed the greatest susceptibility to destruction during processing. Losses of this vitamin appeared to be closely related to the time and temperature of the treatment to which the meats were subjected. Riboflavin was found to be stable under all conditions of processing, and significant losses of nicotinic acid occurred only when chicken meat was smoked and cured. The type of container in which the food was processed appeared to have little influence on retention of vitamins during canning.

Results of determinations for leucine, isoleucine, valine, phenylalanine, tryptophane, threonine, histidine, arginine, and lysine indicated good retention of these amino acids in chicken meat under the studied conditions of cooking and processing. Significant losses were found only with tryptophane. Destruction of this amino acid was uniform in all portions of the meat, and losses occurred regardless of the method of

processing. The losses of leucine, isoleucine, histidine, and arginine resulting from cooking of liver with water suggest the presence of either more readily water-soluble protein constituents in liver than are found in other portions, or the possibility that these amino acids may appear free or loosely combined in the organ. While this portion of chicken meat is not frequently canned or otherwise preserved, losses which may occur are of interest from the standpoint of home preparation by cooking.

The relatively minor losses of the amino acids, with the exception of tryptophane, would indicate that destruction of amino acids is probably not a principal factor in the alteration of the nutritive value of proteins as a result of heating. The processed products would still be good sources of protein insofar as content of the indispensable amino acids is concerned.

SUMMARY AND CONCLUSIONS

An investigation was undertaken to study the effects of cooking, canning, freezing, and curing and smoking processes on the amino acid content and the content of certain of the B vitamins in chicken meat.

Studies on the effect of processing on the thiamine, riboflavin, and nicotinic acid content of chicken meat indicate that significant losses of thiamine occur with all methods of processing, losses being a function of both the time and temperature of the process. Curing and smoking, followed by canning, result in losses of thiamine which may exceed 80 per cent of the original content of this vitamin in fresh chicken meats. Curing and smoking were also found to cause notable losses of nicotinic acid, but no appreciable loss of this vitamin resulted from other methods of processing. No significant loss of riboflavin occurred as a result of any of the processes.

Little destruction of amino acids was noted to occur during commercial processing of chicken meat products, with only tryptophane showing significant losses. As much as 50 per cent of this amino acid may be lost in the canning of chicken meat.

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COMPOSITION OF FOOD PLANTS OF CENTRAL AMERICA
I. HONDURAS

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In developing a food and nutrition program for any country, it is desirable to consider all possible food resources before suggesting the need for importing foods from other areas. Such consideration requires information on the nutritive value, not only of the foods that make up a prominent part of the diet but also of any foods of high nutritive value that are being neglected by the people.

As a preliminary to the establishment of a sound food and nutrition program in the countries of Central America, an investigation is under way in these laboratories to obtain data on the nutritive value of the edible plants found growing in these countries.

One of the investigators (L.O.W.) resides in the area and is responsible for the collection of samples and the botanical identification of the plants. This investigator also prepares stabilized samples for shipment to the laboratories in Cambridge.

The present report includes results from analyses of 121 samples of plant foods collected in Honduras during the last half of 1946 and during March and April of 1947. The following constituents were determined: moisture, ether extract, crude fiber, nitrogen, ash, calcium, phosphorus, iron, carotene, thiamine, riboflavin, niacin, and ascorbic acid.

METHODS OF ANALYSIS

Moisture, ether extract, crude fiber, ash, and calcium were determined by methods of the Association of Official Agricultural Chemists (1945). Nitrogen was determined according to the method described by Hamilton and Simpson (1946). For the determination of total phosphorus a modification of the procedure of Lowry and Lopez (1946) was used. Iron was estimated according to the method described by Hahn (1945).

Carotene was separated chromatographically by a procedure based on the methods of Moore (1940) and Wall and Kelley (1943). The values obtained represent total carotenoid content.

Estimations of thiamine, riboflavin, and niacin were made as soon as possible after samples were received, usually within two to three weeks. Thiamine was measured by the thiochrome procedure and riboflavin was determined fluorometrically. A single extract was used for the measurement of these two constituents, unless the concentration of riboflavin was very high or very low, in which case a second extract was prepared. In making the extract an aliquot of the sample slurry, estimated to contain 10 to 12 micrograms of thiamine, was diluted to 70 ml. with 0.5 per cent

oxalic acid solution and digested in a boiling water bath for 30 minutes. After cooling to room temperature, five ml. of buffered six per cent takadiastase solution was added and the mixture held at 37°C.(98.6°F.) overnight. The solution was then brought to a volume of 100 ml. One aliquot was passed through a column of activated Decalso for thiamine determinations and another through a column of Florisil for riboflavin measurements. Niacin was measured according to the method of the U. S. Pharmacopeia XII (1943).

Ascorbic acid analyses were usually completed within 24 hours of receipt of samples at the laboratory. The method of Roe and Oesterling (1944) for the determination of total ascorbic acid was used. It has been shown by Goldblith and Harris (1948) that values for total ascorbic acid obtained on slurries prepared as described below, and held as long as 10 to 12 days, may be construed as indicating the total ascorbic acid content of the food sample at the time the sample was taken from the fresh plant.

COLLECTION OF SAMPLES

The original plan for this study called for taking samples directly from the growing plant or at least shortly after the product had been harvested. This plan has been followed insofar as possible, but it did not prove feasible for obtaining representative samples of the more commonly used foods. Samples for these were purchased in the markets or directly from growers, as opportunity offered. Histories of the majority of the samples bought in the market were incomplete.

Within a few hours of procurement of a food sample, stabilized samples of the edible portion were prepared for shipment. These samples consisted of slurries made in a Waring blendor. Shortly before slurrying was complete, five ml. of chloroform were added. The prepared samples were then transferred to 300-milliliter brown glass bottles, the caps screwed down tight, and sealed with a pliant sealing wax. The bottles, packed in wooden boxes, were shipped to Cambridge by air-express and usually arrived within two or three days.

For each sample collected in 1946 two slurries were prepared: Sample A consisting of 200 grams of food and 100 grams of 1.0 per cent oxalic acid solution; and Sample B consisting of 100 grams of food and 100 grams of alkaline alcohol solution (30 gm. KOH, 320 gm. ethanol, and water to make 1,000 gm.). Sample A was used for all determinations except carotene. When it became apparent from the work of Goldblith and Harris (1948) that Sample A was not sufficiently acid to stabilize ascorbic acid fully, a third sample, Sample C, was included. This consisted of 50 grams of food and 200 grams of one per cent oxalic acid solution.

A data sheet accompanied each sample on which were recorded the Spanish, English, and scientific names of the food plant, a detailed description of the appearance of the sample, information on altitude, soil type, fertilizer treatment, the estimate for average annual rainfall in the area where the food plant grew, a brief description of local uses of the food, and details of the sampling procedure. A kodachrome was made of representative specimens of each sample. Specimens were also taken for botanical identification and then placed in the herbarium at the Escuela Agrícola Panamericana.

Samples were collected in two areas of Honduras. One area included Tegucigalpa and is representative of the highlands where dry and rainy seasons alternate for approximately six-month periods and the vegetation is sub-tropical. The other area to the north and near the coast is largely lowland and the climate and vegetation are tropical. Information on altitude, soil type, and rainfall in the specific areas where samples were taken is summarized (Table 1).

TABLE 1

Location of Areas Where Samples Were Taken and Information on Altitude, Prevailing Soil Types, and Average Annual Rainfall

Town	Farm	Department	Altitude	Soil type	Rainfall
			ft.		*in. per yr.*
Highland area					
Zamorano	Escuela Agrícola Panamericana	Morazán	2,600-2,700	Sand, loam	40-60
Tegucigalpa	La Granja	Morazán	3,150	.....	40-60
Jicarito	.....	Morazán	2,900	.....	40-60
Guinope	.....	El Paraíso	.....	Loam, sand	40-60
Danlí	.....	El Paraíso	.....	Loam, sand	40-60
Yuscarán	.....	El Paraíso	3,600	Loam, sandy loam	.....
Lowland coastal area					
La Lima	Research Division Gardens, Tela Railroad Co.	Cortés	300	Loam	50±
La Lima	.....	Cortés	100	Loam, sand	50±
Las Mesas[1]	.....	Cortés	300	Loam, sand	50±
San Pedro Sula	.....	Cortés	100	Loam, sandy loam	50-60
San Juan Lindo[2]	.....	Cortés	3,000	.....	
Puerto Cortés	.....	Cortés	0	Sandy	50±
Near Tela	Lancetilla Experimental Garden	Atlantida	100	Clay, sand, clay loam	100±

[1] Near La Lima. [2] Above San Pedro Sula.

DESCRIPTIONS AND USES OF FOODS STUDIED

EARTH VEGETABLES:

Camote. Sweet potatoes are fairly common in the dietary of Central Americans and several varieties are to be found in most markets. The sample represents the Nancy Hall variety, known in Guatemala as the "Zamorano."

Cebolla. The sample of shallot consisted of whole bulbs taken from drying bins.

Cebolla. Onions are used as a cooked vegetable or as a condiment. The onions for Sample (a) were mature bulbs and were taken from drying bins. Those for Sample (b), purchased in the market, had green tops but were nearly mature.

Jícama. This root is irregular in shape and has a white skin and white, watery flesh. It is used as a cooked vegetable or eaten raw. The sample consisted of five peeled roots.

Malanga. The Xanthosomas are native to tropical America, are grown in wet places, and may be used in soups or as a cooked vegetable. Cooking destroys the poisonous principle they contain.

Papa. Potatoes are undoubtedly native to this hemisphere, but are not a staple food in Central America. Production, carried on mostly in the highlands, is somewhat haphazard and the potatoes are usually small and of poor quality. Most commonly they are used mixed with other vegetables or in stews.

Puerro. The sample of leeks for this study consisted of the blanched part of the bulb.

Rábano. Radishes are commonly cultivated in the highlands of Central America, where they do well. They are usually found on sale in the larger markets. Generally, they are eaten raw or they may be cooked.

Yuca. Cassava is an important food plant in the tropics although it is not used as extensively in Central America as in some other regions. Probably this is because maize is more popular. Cassava is used as a vegetable, cooked in various ways. The flesh of the roots used for Sample (b) had a yellow tinge.

Zanahoria. The carrot is appreciated in Central America as much as any of the vegetables and is eaten cooked or raw. In season carrots are found in practically all markets, although the price may vary considerably according to their abundance. Peeled, whole carrots were used in the sample.

HERBAGE VEGETABLES:

Acelga. Chard is seldom grown in Central America, although it is found occasionally in the large markets when in season. Mature leaves made up the sample.

Brocoli. Broccoli is grown to a limited extent in Central America. The sample came from an experimental plot and consisted of the terminal growth (stem, leaf, and flower) from several plants.

Camote, hojas de. So far as is known the leaves of the sweet potato plant are not used as food in Honduras. The sample for analysis was prepared from the tender young terminal growths of plants of the Nancy Hall variety.

Chaya. This tree is grown as an ornamental in some areas of Honduras. The sample was prepared from the leaves, which are edible, although they are not known to be used as food.

Coliflor. Cauliflower can be grown readily in highland areas of Central America, but it is not very common. The sample consisted of the heads only.

Colinabo. Kohlrabi is not cultivated extensively in Central America. The sample was made from peeled, mature stems from which the leaves had been removed.

Espinaca. New Zealand spinach is native to New Zealand and has become popular as a food plant rather recently. It is found infrequently in the markets in Central America. It should become an important food plant, as it withstands the dry season well. The edible portion consists of the new growth, including tender leaves and stems.

Espinaca. This plant is a small, succulent herb, native to Central America, from whence it was carried to the Philippine Islands, where

it is appreciated as a food plant. In Central America it is most often found growing as a weed.

Jilote. Indian corn or maize originated in this hemisphere and is one of the most important food crops in Central America. Varieties have been developed that withstand extremes of rainfall, altitude, heat, and soil conditions. It is doubtful whether the relatively dense populations of the cool tropical highlands could easily survive without maize. From the time the ears begin to form, until the grains are mature, it serves as food. In the immature state the whole ear *(jilote),* including the cob and sometimes the tender inner husks, may be eaten boiled as a vegetable or in soups and stews. As the grain matures further it is prepared as boiled green corn and only the kernels are eaten. At this stage the grains may be cut from the cob and used to make *tortillas.* Finally, the fully mature corn is dried and stored for use throughout the year in making *tortillas* and *tamales.* The sample of *jilote* was prepared from about 100 tender ears after the husks were removed.

Lechuga. Lettuce is found infrequently in markets of Honduras and is usually poor in quality and high in price. For sampling, the leaves were washed, wiped dry, chopped, and mixed.

Repollo. Cabbage grows well in the highlands of Central America and poorly in the wet or dry coastal plains. In season it is abundant in most markets and is relatively cheap. Sample (a) was a flat-headed variety. Sample (b) consisted of heads of the common green cabbage, which were poor in quality but expensive. The heads used in Sample (c) were of poor quality, also. All samples were prepared by taking a quarter from several heads, and chopping and mixing.

Repollo Chino. Chinese or celery cabbage is used in Central America as a cooked or a green vegetable, but is found in the markets infrequently.

Rosa de Jamaica. This plant is more important in Central America as a source of fiber than as a source of food. The dark-red, fleshy, accrescent calyces, Sample (a), are used to make jelly similar to cranberry jelly. The young ovaries, Sample (b), may be used as a cooked vegetable. The two samples were made from the same flowers.

Santa María. This plant is widely distributed in Central America, especially in the Atlantic lowlands where it is native. The fresh leaves have an odor resembling sarsaparilla and are used as flavoring in soups or stews. The sample consisted of tender, new leaves.

Tampala. This plant, related to one of the pigweeds, is most often found growing as a weed. When eaten in Central America it is prepared as a cooked vegetable.

Verdolaga. Purslane is widely distributed in Central America from the coast up into the highlands. It is found most often growing as a weed. When eaten it is cooked like spinach.

Yuca blanca, hojas de. It is not known that the leaves of the cassava are used as food in Central America. In any case they should not be eaten unless cooked because of the toxic principle they contain.

Heteranthera reniformis Ruiz and Pavón. This edible herb is not known to be used as a food in Honduras.

Jussiaea repens L. The plants used for this sample grew in and on the banks of the Río Yeguare. Only the apical leaves and part of the stems were used.

FRUIT VEGETABLES:

Aguacate. The avocado is native to Mexico and Central America and is an important food plant in these areas. The Guatemalan and West Indian races, classified botanically as *Persea americana*, are the ones cultivated most commonly in Central America. The fruits, which have a thick and often shell-like skin, may be eaten as a vegetable, raw on salads, or as a fruit out of hand.

Ayote. Squashes and pumpkins are important in the Central American dietary and even the seeds are used. The fruits are used cooked as a vegetable, in soups, and sometimes with meat. The seeds are dried, roasted, and eaten like nuts. Sample (a) of *ayote* was described as having pale-green-to-white flesh and a very thin skin. Both seeds and flesh are eaten and the whole fruit was included in the sample. Sample (b) consisted of a goose-necked and oblong-ovoid fruit, having a soft skin. The prepared sample was made from the flesh only. The common pumpkin, *ayote comun*, has a hard, shell-like skin and firm, yellowish flesh. The sample included the flesh only. *Chiberro* resembles a pumpkin. The immature fruit has white pulp and seeds and is eaten as a boiled vegetable. The flavor is rather bland. A sweet, *alcitron*, is made from the flesh. The black seeds from mature fruits are used to make a drink. The sample was prepared from the skin and pulp of immature fruits. *Pipián* is a small squash with a soft skin and white or cream flesh. Whole fruits were used for the sample.

Berengena. The eggplant is cultivated easily in the tropical highlands and with reasonable care produces an abundance of fruit. In season, this vegetable is found in most of the larger markets, but the price is high.

Chiberro. See under *Ayote.*

Chile dulce. Sweet peppers are used rather generally in Central America and are eaten cooked or raw. They may be found in most markets in season but usually not in abundance. Sample (a) consisted of ripe (red) fruits and Sample (b) of green fruits with some red coloration.

Cojombro. The flesh of this immature gourd is white, moist, and spongy, and as a vegetable it is inferior. The gourds are generally allowed to mature so that the very durable skin or "shell" may be used for containers of liquids or foods. The sample for analysis consisted of the soft pulp and seeds of immature fruits.

Miltomate. The cherry tomato is native to tropical America and may be the progenitor of the cultivated tomato. It is found in many markets in Honduras from August to January and is usually inexpensive. It is eaten raw in salads or cooked in soups, stews, or with meat. The whole berries were used in the sample.

Ocra. This food plant is fairly common in Central America and usually is very cheap. It is used as a cooked vegetable and in soups and stews. Whole fruits were used for Sample (a), while Sample (b) was prepared by taking cross sections from several fruits.

Pataste. Chayotes may have originated in Central America, but they are now grown throughout the tropics of the world, usually cultivated as door-yard plants. They grow with little care and produce an abundance of fruits which are used as a vegetable. Other parts of the plant may be eaten also: the roots as a cooked vegetable, the tender leaves as greens, and the flowers in egg dishes. The fruits are cheap and are found in almost all markets during most of the year.

Pepino. The cucumber is not an important vegetable in Central America. Green fruits may be eaten raw as salad, and green or ripe fruits as a cooked vegetable.

Pipián. See under *Ayote.*

Tomate. Tomatoes are cultivated quite commonly in Central America. In season they are plentiful in most markets and at peak season are relatively cheap. Sample (a) comprised rather small fruits. The fruits for Sample (b) were taken from the vines and ripened in the house for two days. Only the pulp and skin were used. The fruits used for Sample (c) were fully ripe (red) and the whole fruit (skin, pulp, and seeds) was included.

LEGUMES:

Frijol de vaca. Cowpeas are widely cultivated in tropical and subtropical regions. The plant furnishes food for stock as well as for human beings. Although the dry seeds, as well as the green pods, are edible, only the green pods are eaten in Central America.

Gandul. The pigeon pea is a food of considerable potentiality. The plant is a shrub that adapts itself to most tropical climates, requires little care in cultivation, and continues to produce food even during the dry season. The seeds may be eaten as a vegetable when they are green or after they are mature and dry. The green peas are difficult to shell. Immature seeds made up the sample.

Guajiniquil. The tree bearing this fruit is frequently used as shade for coffee trees. The soft, velvety, succulent covering of the seeds is eaten raw. The bean, which is deep green in color, is eaten after prolonged boiling, although it is still tough and leathery. The bean and the soft covering were used in the sample.

Haba. Lima beans are probably native to this hemisphere. The sample consisted of immature seeds from pods selected in the field.

Habichuela. The kidney bean originated in this hemisphere and includes the common varieties of beans used as shell or snap beans. Next to maize, beans are the most popular staple food in the Central American dietary. Snap beans and fresh-shelled immature beans are prepared in a variety of ways. The dry beans are commonly boiled, then mashed and fried. In Honduras the red bean is most popular. Green string or snap beans appear in most markets, but because of their seasonal nature and the reluctance of the native people to eat greens, their place in the dietary is of lesser importance than that of dried beans.

Paterna. The leguminous tree on which this fruit grows is used extensively as shade for coffee trees. The large seeds are covered with a juicy, sweet white pulp which is eaten raw. The seeds are eaten after cooking.

The sample was prepared from legumes in which the seeds were sprouting and included the edible seed, the soft white covering of the seeds, and the sprouts.

Canavalia ensiformis (L.) DC. This legume is seldom cultivated in Central America and has not been seen in any market. The large pods which are produced in abundance may be used while immature. The ripe beans which are surrounded by a sweet aril, may be used much as are green lima beans.

FRUITS:

1. *Pome Fruits*

Icaco. This apple-like fruit grows on a small tree (shrub) found in coastal thickets. The fruits are small and have a thin skin which is pink-to-black in color. The flesh is white and spongy and has an insipid flavor. The sample was prepared from the peel and pulp.

Membrillo. Quinces are not abundant in Honduras. Generally, they are eaten as a fruit out of hand.

Perote. Apples do not grow well in the tropics and are usually small and of inferior quality. They are used raw or cooked.

2. *Stone Fruits*

Ciruela tronadora. This rather important fruit tree is a native of Central America. It is easily grown and widely distributed. In season the fruits are found in most markets. Most commonly they are eaten out of hand from the stage at which the flesh begins to soften to that at which the fruits are fully ripe (red or yellow). Fruits from various sources differ considerably in quality.

Coco. In the world economy coconuts are among the most important of plants. The chief commercial raw product is copra, from which coconut oil is extracted. The coconut is an immediate source of food in localities where it grows. Disease and beetle attacks threaten extermination of the coconut palm in Central America. The sample was prepared from the flesh of ripe fruits.

Coco, agua de. (See under *Coco.*) The sample consisted of the "milk" from the fruits used in preparing the sample of coconut "meat" described above.

Coyol. The flavor of the pericarp of this fruit is not agreeable and this restricts its use as food. The sample consisted of the pericarp and endocarp from several fruits.

Jambolana. This fruit is rare in Central America. For the prepared sample the seeds were removed.

Mango. The mango is perhaps the fruit tree most widely planted in tropical and sub-tropical lands. It is believed to have been brought to Brazil by the Portuguese at the beginning of the 18th Century or earlier. From there it was brought to the West Indies and thence it scattered into Central America. The fruit varies widely in quality with variety. Fruit of seedling trees tends to be fibrous and of poor flavor. Grafted varieties with low fiber content and excellent flavor are now being propagated in the sub-tropics as well as in the tropics. Sample (a) consisted of good-

sized fruits which were ripened after purchase. The fruits of Sample (b) were small, rather fibrous but of fair flavor and presumably tree-ripened. The fruits for Sample (c) were small, elongated, and sigmoid, and had an attractive rich-apricot coloring. This highly prized variety has few fibers and is the most expensive.

Manzana rosa. Both the flowers and the fruit of this plant are eaten. The flowers, which are large, are folded into egg dishes. The fruits are rather dry but may be eaten raw. The sample comprised mature fruits.

Pejibaye. The flesh surrounding the seed of the fruit of this palm is much appreciated as food and is eaten either boiled or roasted. The prepared samples consisted of the flesh separated from the seed. Sample (a) was from a carrot-colored variety, while Sample (b) was pale yellow.

3. *Berries*

Aberia. This fruit is produced in abundance on a shrub. It is rarely grown in Central America as the fruits are very acid and therefore little appreciated. The berries make fine jelly or jam. The sample was prepared from the whole berries.

Chachalaca. The small tree producing these berries grows along river banks. The fruit, which is extremely sweet and juicy, is not cherished to any extent, except by small boys and birds, unless there is extreme food shortage.

Sarsil. The shrub on which this berry grows is somewhat similar to the blueberry and is used extensively in hedges or as an ornamental. The berries, which are produced in enormous quantities, have a tart flavor and sometimes are used in conserves or occasionally as fresh fruit. Entire fruits were used for the sample.

Zarzamora. This blackberry, a new species, is native to Honduras and sometimes is in semi-cultivation in the highlands. The fruits, which have large seeds, are slightly acid and make excellent jams and jellies. The sample was collected from brambles growing on a fence, and although the berries were ripe and children lived nearby, there was no indication that berries had been picked.

4. *Citrus Fruits*

Lima or Limón. This lime is larger than the common variety. Although plantings are not uncommon, limes are not found often in markets of Central America. They are eaten as a fruit out of hand or used in beverages.

Lima limón. The sweet lime, possibly a native of India, is widely distributed in Latin America and is one of the more aporeciated of the secondary citrus fruits. The ripe fruit is pale green an ' has abundant juice which is slightly bitter and somewhat insipid to the taste. It is used as a fruit out of hand or in beverages.

Limón. Lemons are not commonly cultivated in Central America and are found infrequently in the markets. Though smaller than those grown in the United States they have a finer flavor.

Limón ponderosa. This furit is not commonly cultivated in Central America. The tree might be grown as an ornamental and the fruit used

for home consumption. The juice may be used in cooking or for beverages.

Mandarina. In season, tangerines are found in most good markets in Central America. They are often of indifferent quality, however. The samples for analysis were prepared from the pulp, with the seeds removed.

Toronja. This acid fruit is not greatly appreciated by Latin American peoples. No attempt has been made to cultivate the superior varieties, and the fruits available are usually of inferior quality.

5. *Bananas*

Banano. The banana is the most important and familiar of tropical fruits. It grows throughout the tropics where there is sufficient rainfall to support the plants, and unlike most fruits, is best when ripened after harvesting.

Sample (a) was one of the small-fruited varieties, or finger bananas. These are not widely cultivated. The skin of the fruit is thin and the flesh firm and dry. When green this variety is eaten boiled, roasted, or baked, and when ripe is eaten as a fruit. Sample (b) was from fully ripe fruits of the Gros Michel variety, the variety commonly shipped to the United States. Sample (c) represents fruits of the variety "Raja." These fruits are relatively short and thick, and the flesh is cream-yellow, moist, sweet and strong-flavored. This variety is not cultivated to any extent. Sample (d), "Pisang seraeanota," was in the ripening room seven days. This variety is not cultivated to any extent. Sample (e), "Pisang Mangson," was held in the ripening room seven days. The fruits of this variety, which is not grown commonly, are small, thin-skinned, with pale, somewhat dry, flesh of inferior flavor. The fruits for Sample (f), Lacatan, were held in the ripening room 10 days. This variety is one of the more desirable types of banana. Sample (g), Giant Fig, also was held in the ripening room 10 days.

Plátano. The plantain is an important food plant in most tropical and sub-tropical regions, including Central America. There are some 75 recorded varieties. In Honduras a number of names are used that may apply to more than one variety. Names noted for the variety represented by Sample (a) are *plátano, cuadrado, majoncho chato, butaco,* and *moroca.* Samples (e), (f), and (g) represent the large plantains known as horse plantains. Most plantains are cooked when green or ripe by boiling, roasting, frying, boiling and frying, or boiling and roasting. Cooked plantains may be eaten as a vegetable or in a dessert. When fully ripe some plantains may be eaten raw.

The fruits of Sample (b) were mature, yellow, and relatively hard. Fruits of Sample (c), *cenizo apple plantain,* and Sample (d), *da Costa white,* were thick with white flesh. The three samples of large plantains varied considerably in appearance. The fruits of Sample (e) had relatively thin skins and yellowish flesh. These fruits are soft when ripe. Fruits for Sample (f), ripened five days, had yellow skins and cream flesh which was rather dry. Sample (g) comprised fully ripe fruits. This plantain is easily grown. It is cheap and probably one of the most important varieties.

6. *Other Fruits*

Anona blanca. The sugar apple is greatly appreciated in Central America for its fine sweet flavor and is found commonly in the markets. The sample consisted of the flesh from fruits picked green and ripened for two days.

Caimito. This fruit is one of the best of the Sapotaceae and good variations are among the best of tropical fruits. In addition, the parent tree makes a handsome shade tree. For the prepared sample the fruits were peeled and the seeds removed.

Carambola. The carambola is not common in Central America and is rarely found in the market. It may be used as a fruit, in preserves, or to make vinegar. The sample was prepared from the flesh and skin of mature fruits.

Cola. The cola nut is said to contain about two per cent of caffeine and kolanin, a heart stimulant. The tree is a native of West Africa, where the nut is used by the natives as a masticatory. The tree is rare in Central America. Extracts of the nut are used in syrups for soft drinks.

Durio. Although this fruit is greatly appreciated by those accustomed to its "spoiled garlic" odor, it is rarely found in Central America. The covering of the seed is cooked and eaten like a vegetable. The seeds are eaten roasted. The sample was prepared from the seed covering.

Higo. This fruit is rather insipid and is rarely used. Deer are said to relish it. The sample was prepared from the fleshy part of the fruit.

Níspero. The sapodilla is a highly prized fruit in Central America and is found rather commonly at middle and lower elevations. Chicle is the product for which the tree is best known in other regions. The sample was prepared from the greenish-yellow flesh of about 50 small fruits taken from trees.

Piña. The pineapple can be propagated easily in Central America and is appreciated for its fine flavor. However, it is not grown extensively, the fruits produced are usually inferior, and the market price is usually high. The variety *Piña de agua,* Sample (a), is small-fruited and is used as a fresh fruit and in conserves. Mature fruits were taken for the sample. The fruits of the variety *Piña de azúcar,* Sample (b), are also small, rather sweet, and have abundant juice which is used as a beverage. The fruits used for Sample (c) represent a large-fruited variety. Fruits of this variety may be used as fresh fruit, in preserves, or for juice. The fruits for the sample were ripened off the plant.

Zapote or Sapote. The *sapote* is probably of Central American origin. It is considered among the best of tropical fruits and is an important adjunct to the dietary. In season it is found in practically all markets. It is eaten as a fruit out of hand and may be used in preserves and desserts. The *sapote* may be distinguished from the *mamey* by its shiny black seeds.

RESULTS AND DISCUSSION

Results from the analyses of the 121 samples are shown (Table 2). To aid in interpretation, these data are presented with the food items grouped under three main headings—vegetables, legumes, and fruits. All values are expressed per 100 grams of the raw, edible material.

Ascorbic Acid-Total
mg.
35.0
0.8
7.5
6.7
5.5
7.0
16.8
17.3
7.8
10.0
30.8
48.2
35.4
7.5
42.5
78.0
38.2
175.5
32.0

A number of foods are represented by two or more samples and it is of interest to compare their composition.

Cebolla. The two samples, (a) and (b), were remarkably similar in composition although they came from different localities and were not equally mature.

Rábano. The main difference in the two samples was in their content of ascorbic acid (10.0 and 30.8 mg.).

Yuca. The two representatives showed variations in content of nitrogen (0.083 and 0.215 mg.) and phosphorus (20.2 and 74.8 mg.).

Papa. Like *cebolla*, the two samples of potato were remarkably similar in composition.

Espinaca. Sample (a) of the New Zealand spinach consisted of mature, deep-green leaves, while Sample (b) was made up from the shoots of tender, new plants started in the rainy season. These samples showed small differences in content of carotene (1.501 and 0.513 mg.) and riboflavin (0.175 and 0.098 mg.).

Repollo. None of the three samples of cabbage was of first quality. They were quite similar except in content of ether-extractable material (1.18, 0.29, and 0.13 mg.) and carotene (0.038, 0.004, and 0.007 mg.).

Verdolaga. The three samples of purslane varied erratically in composition. Samples (a) and (b) were prepared from the leaves and stems of mature plants and Sample (c) from the tender parts of stems and leaves.

Ayote. The two samples of squash came from localities representing extremes of altitude but, like *cebolla* and *papa*, differed little in composition.

Chile dulce. The two samples of sweet pepper showed pronounced variations in content of carotene (1.167 and 0.115 mg.), riboflavin (0.142 and 0.036 mg.), and ascorbic acid (177.8 and 99.0 mg.). The fruits of Sample (a) were ripe (red), while those of Sample (b) were green with some red coloration.

Miltomate. The two samples showed only minor differences, the greatest being in carotene content (0.942 and 0.545 mg.).

Ocra. Sample (a) was lower than Sample (b) in content of calcium (58.9 versus 114.8 mg.), phosphorus (32.9 versus 73.6 mg.), and niacin (0.932 versus 1.550 mg.), but higher in content of carotene (0.042 versus 0.006 mg.) and of ascorbic acid (39.2 versus 18.6 mg.). Entire fruits were used for Sample (a), while for Sample (b) cross sections from several fruits were used.

Pataste. The two samples showed no significant differences in nutrient content.

Pepino. The samples of cucumber showed insignificant differences in composition.

Tomate. The three samples of tomato showed minor differences in content of niacin (0.400, 0.470, and 0.710 mg.) and ascorbic acid (13.9, 24.0, and 9.9 mg). Samples (a) and (b) comprised skin and flesh, while Sample (c) contained seeds, also.

Habichuela. Two samples were analyzed. Sample (a) was taken directly from the plants, while Sample (b) was purchased in the market. Sample (a) had a higher content of calcium (53.0 versus 36.9 mg.), caro-

56.6
28.6
32.8
53.8
9.7
4.7
54.5
53.0
41.3
24.0
14.4
22.9
70.0
86.4
16.7
22.1
24.5
320.5
16.0
86.9
11.8
14.8
11.3
14.0

TABLE 2

Composition of Food Samples From Honduras

Name of food sampled			Place sample obtained [2]	Constituents measured and yields per 100 grams												
Spanish [1]	Scientific	English		Moisture	Ether extract	Crude fiber	Nitrogen	Ash	Calcium	Phosphorus	Iron	Carotene	Thiamine	Riboflavin	Niacin	Ascorbic Acid-Total
	EARTH VEGETABLES			*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Camote	*Ipomoea Batatas (L.)* Lam.	Sweet potato	[2]Escuela	71.1	0.10	0.1	0.193	.93	25.9	19.2	1.58	2.040	.092	.029	.483	35.0
Cebolla	*Allium ascalonicum*	Shallot	[2]Escuela	79.8	<0.10	0.7	0.405	.83	36.6	59.5	1.25	.006	.060	.020	.180	0.8
Cebolla (a)	*Allium Cepa L.*	Onion	[2]Escuela	93.0	0.10	0.6	0.205	....	....	....	....	.003	.032	.024	.185	7.5
Cebolla (b)	*Allium Cepa L.*	Onion	San Juan Lindo	83.8	0.10	0.6	0.348	.61	20.2	54.7	0.42	.000	.053	.020	.434	6.7
Jícama	*Pachyrrhizus erosus (L.)* Urban	Yam bean root	La Lima	89.5	0.12	0.5	0.157	.23	17.1	13.0	0.54	.000	.033	.016	.247	5.5
Malanga	*Xanthosoma violaceum* Schott	.....	San Pedro Sula	58.3	0.41	0.5	0.306	.87	8.3	55.9	0.34	.002	.145	.022	.635	7.0
Papa (a)	*Solanum tuberosum L.*	Potato	Jicarito	80.7	<0.10	0.4	0.263	.92	7.3	28.3	0.82	.000	.077	.026	1.072	16.8
Papa colorada o roja (b)	*Solanum tuberosum L.*	Potato	San Juan Lindo	75.8	0.08	0.3	0.355	.87	6.1	53.7	0.93	.004	.103	.015	1.340	17.3
Puerro	*Allium Porrum L.*	Leek	[2]Escuela	84.9	0.07	1.2	0.347	.97	69.8	58.7	1.46	.014	.103	.052	.579	7.8
Rábano (a)	*Raphanus sativus L.*	Radish, Early Scarlet Globe	[2]Escuela	93.0	0.10	1.7	0.184	....	....	....	....	.004	.025	.035	.570	10.0
Rábano (b)	*Raphanus sativus L.*	Radish	San Juan Lindo	94.1	0.09	0.7	0.178	.91	26.4	28.7	1.02	.002	.024	.017	.368	30.8
Yuca (a)	*Manihot esculenta* Crantz	Manioc or Cassava	[2]Escuela	58.5	0.13	0.8	0.083	.93	48.2	20.2	0.78	.006	.054	.024	.491	48.2
Yuca dulce (b)	*Manihot esculenta* Crantz	Manioc or Cassava	La Lima	55.4	0.23	1.4	0.215	1.56	36.7	74.8	0.62	.010	.040	.029	.737	35.4
Zanahoria	*Daucus Carota L.*	Carrot	[2]Escuela	87.2	0.42	0.9	0.244	1.04	33.5	72.5	1.13	5.650	.058	.050	.903	7.5
	HERBAGE VEGETABLES															
Acelga	*Beta vulgaris* var. *cicla* Moq.	Swiss chard	[2]Escuela	90.6	0.52	0.8	0.481	.97	77.4	49.3	2.72	3.020	.108	.281	.599	42.5
Brocoli	*Brassica oleracea* var. *botrytis L.*	Broccoli	[2]Escuela	86.3	1.18	1.3	0.747	1.03	97.5	90.6	1.26	1.627	.177	.239	1.681	78.0
Camote, hojas de	*Ipomoea Batatas (L.) Lam.*	Sweet potato leaves	[2]Escuela	87.8	0.66	1.3	0.410	1.02	98.1	27.6	3.03	3.340	.068	.208	.699	38.2
Chaya	*Cnidoscolus aconitifolius* (Mill.) I. M. Johnston	.....	[2]La Lima	76.4	1.55	2.3	1.300	2.60	333.6	81.9	10.77	5.960	.222	.526	2.000	175.5
Coliflor	*Brassica oleracea* var. *botrytis L.*	Cauliflower	[2]Escuela	86.4	0.06	0.9	0.759	0.95	48.8	71.0	0.99	.039	.141	.125	.800	32.0

Colinabo	*Brassica caulorapa (DC.)* Pasq.	Kohlrabi	[2]Escuela	91.9	0.10	1.2	0.265	....	....	....	....	.007	.058	.039	.380	56.6
Espinaca (a)	*Tetragonia expansa* Thunb.	New Zealand spinach	[2]Escuela	90.8	1.12	0.7	0.392	1.80	84.0	33.0	1.75	1.501	.018	.175	.588	28.6
Espinaca (b)	*Tetragonia expansa* Thunb.	New Zealand spinach	[2]Escuela	93.9	0.32	0.5	0.417	1.31	41.7	28.4	1.50	.513	.007	.098	.481	32.8
Espinaca	*Talinum triangulare* (Jacq.) Willd.	Philippine spinach	[2]La Lima	90.3	0.56	0.8	0.426	2.27	120.1	26.9	5.49	.041	.096	.174	.402	53.8
Jilote	*Zea Mays L.*	Corn, immature	Danlí	88.9	<0.10	0.6	0.282	.61	10.1	57.0	0.45	.003	.069	.066	.600	9.7
Lechuga	*Lactuca sativa* var. *Romana Hort.*	Lettuce	San Juan Lindo	95.5	0.27	0.5	0.191	.80	42.6	23.2	1.44	.014	.058	.075	.428	4.7
Repollo (a)	*Brassica oleracea* var. *capitata L.*	Cabbage	[2]Escuela	91.9	1.18	1.1	0.291	.67	69.6	38.8	0.72	.038	.053	.038	.336	54.5
Repollo (b)	*Brassica oleracea* var. *capitata L.*	Cabbage	[2]La Lima	90.7	0.29	0.9	0.370	.83	47.8	43.9	0.58	.004	.052	.048	.335	53.0
Repollo (c)	*Brassica oleracea* var. *capitata L.*	Cabbage	[2]Lancetilla	91.8	0.13	0.8	0.324	.70	62.2	26.7	0.35	.007	.051	.043	.374	41.3
Repollo Chino	*Brassica pekinensis* (Lour.) Rupr.	Chinese cabbage	[2]Escuela	95.5	1.28	0.7	0.160	.75	69.0	23.5	2.47	.158	.030	.034	.450	24.0
Rosa de Jamaica (a)	*Hibiscus Sabadariffa L.*	Roselle	[2]Escuela	89.9	1.35	1.0	0.122	.52	70.9	16.9	3.08	.006	.014	.035	.340	14.4
Rosa de Jamaica (b)	*Hibiscus Sabadariffa L.*	Roselle	[2]Escuela	75.5	1.54	5.9	0.564	.97	103.0	62.8	1.11	.045	.089	.085	1.220	22.9
Santa María	*Piper auritum HBK.*	.....	[2]La Lima	83.8	1.07	1.6	0.573	2.36	197.4	54.0	6.54	1.910	.110	.204	.933	70.0
Tampala	*Amaranthus gangeticus L.*	Tampala	[2]Escuela	84.8	0.29	1.3	0.669	2.82	578.0	92.3	3.55	1.801	.002	.270	2.288	86.4
Verdolaga (a)	*Portulaca oleracea L.*	Purslane	[2]Escuela	91.5	0.29	1.0	0.275	....	....	....	....	.898	.031	.092	.543	16.7
Verdolaga (b)	*Portulaca oleracea L.*	Purslane	[2]Escuela	92.1	1.11	0.7	0.251	1.40	82.5	27.4	7.20	.970	.017	.106	.469	22.1
Verdolaga (c)	*Portulaca oleracea L.*	Purslane	[2]La Lima	87.8	0.45	1.3	0.356	2.11	137.8	38.5	2.85	.060	.020	.080	.547	24.5
Yuca blanca, hojas de	*Manihot esculenta* Crantz.	Manioc leaves	[2]Research Div. garden	96.7	3.02	2.3	1.690	2.45	292.2	113.1	8.08	6.520	.272	.425	3.530	320.5
	Heteranthera reniformis Ruiz and Pavón	An herb	[2]Río Yeguare near Zamorano	93.8	0.19	0.8	0.153	1.12	63.1	37.1	18.47	1.673	.031	.083	.260	16.0
	Jussiaea repens L.	Aquatic herb	[2]Río Yeguare near Zamorano	87.5	1.04	0.8	0.402	1.14	144.2	65.3	8.03	3.940	.043	.118	.836	86.9
		FRUIT VEGETABLES														
Aguacate	*Persea americana* Mill.	Avocado	Jicarito	74.5	16.69	1.5	0.206	.80	15.6	30.9	1.24	.182	.051	.093	1.667	11.8
Ayote (a)	*Cucurbita Pepo L.*	Pumpkin	[2]Escuela	94.6	0.33	0.3	0.104	.52	26.0	25.2	0.65	.019	.027	.032	.440	14.8
Ayote (b)	*Cucurbita Pepo L.*	Pumpkin	La Lima	93.9	0.24	0.5	0.189	.52	30.7	34.3	0.41	.007	.047	.034	.351	11.3
Ayote común	*Cucurbita Pepo L.*	Pumpkin	Danlí	84.7	0.22	0.8	0.122	.68	16.1	42.7	0.73	.142	.076	.040	.700	14.0

TABLE 2 *(Continued)*

Composition of Food Samples From Honduras

Name of food sampled			Place sample obtained [2]	Constituents measured and yields per 100 grams												
Spanish [1]	Scientific	English		Moisture	Ether extract	Crude fiber	Nitrogen	Ash	Calcium	Phosphorus	Iron	Carotene	Thiamine	Riboflavin	Niacin	Ascorbic Acid-Total
				gm.	*gm.*	*gm.*	*gm.*	*gm.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Berengena	*Solanum Melongena* var. *esculentum* (Dun.) Nees	Eggplant	[2]Escuela	92.3	0.10	0.7	0.194	.49	11.3	17.9	1.25	.011	.028	.034	.483	5.0
Chiberro	*Cucurbita Pepo L.*	Pumpkin	Yuscarán	94.7	0.11	0.4	0.111	.34	12.5	28.7	0.67	.000	.019	.026	.323	18.4
Chile dulce (a)	*Capsicum annuum* var. *grossum (L.)* Sendt.	Sweet pepper	[2]Escuela	90.0	0.45	0.9	0.168	....	....	....	....	1.167	.060	.142	.815	177.8
Chile dulce (b)	*Capsicum annuum* var. *grossum (L.)* Sendt.	Sweet pepper	[2]Escuela	92.2	0.36	0.8	0.135	.42	6.5	26.5	0.98	.115	.061	.036	.585	99.0
Cojombro	*Lagenaria siceraria* (Mol.) Standl.	Gourd or calabash squash	[2]Escuela	92.2	0.21	1.5	0.111	.59	17.0	26.5	0.61	.004	.040	.027	.578	19.4
Miltomate (a)	*Lycopersicum esculentum* var. *cerasiforme* (Dunal) Hort.	Cherry tomato	Danlí	90.0	0.64	1.1	0.286	.78	13.1	39.8	0.63	.942	.160	.073	.880	38.0
Miltomate o Tomatillo (b)	*Lycopersicum esculentum* var. *cerasiforme* (Dunal) Hort.	Cherry tomato	La Lima	89.2	0.34	1.8	0.314	.88	17.7	25.6	0.49	.545	.108	.098	.850	33.7
Oera (a)	*Hibiscus esculentus L.*	Okra	[2]Escuela	90.2	0.43	0.6	0.330	.75	58.9	32.9	1.14	.042	.088	.059	.932	39.2
Oera (b)	*Hibiscus esculentus L.*	Okra	San Pedro Sula	85.6	0.37	1.0	0.354	1.04	114.8	73.6	1.25	.006	.098	.060	1.550	18.6
Pataste (a)	*Sechium edule Sw.*	Chayote	Guinope.	93.4	0.16	0.4	0.088	.39	16.3	18.7	0.42	.041	.018	.023	.343	14.4
Pataste (b)	*Sechium edule Sw.*	Chayote	Yuscarán	90.1	0.05	0.4	0.183	.60	11.0	44.0	0.73	.001	.028	.032	.651	19.9
Pepino (a)	*Cucumis sativus L.*	Cucumber	[2]Escuela	95.2	<0.10	0.3	0.092	.39	19.7	20.7	0.44	.013	.023	.026	.196	16.4
Pepino (b)	*Cucumis sativus L.*	Cucumber	Las Mesas	91.5	0.16	0.5	0.122	.53	19.4	24.8	0.52	.032	.040	.020	.612	7.0
Pipián	*Cucurbita Pepo L.*	Squash	La Lima	92.6	0.07	0.3	0.182	.60	24.4	38.9	0.51	.006	.056	.043	.533	14.0
Tomate (a)	*Lycopersicum esculentum (L.)* Mill.	Tomato	[2]Escuela	94.9	0.23	0.5	0.167	....	....	....	....	.313	.067	.028	.400	13.9
Tomate (b)	*Lycopersicum esculentum (L.)* Mill.	Tomato	[2]Escuela	94.0	0.29	0.5	0.137	.50	9.8	26.5	1.02	.312	.071	.030	.470	24.0
Tomate (c)	*Lycopersicum esculentum (L.)* Mill.	Tomato	San Pedro Sula	94.7	0.30	0.6	0.180	.48	6.2	27.7	0.80	.305	.086	.030	.710	9.9
		LEGUMES														
Frijol de vaca o Frijol de ojo negro	*Vigna unguiculata (L.)* Walp.	Cowpea var. "Victor"	[2]La Lima	78.3	0.45	3.2	0.800	0.97	53.2	80.5	1.24	.025	.168	.026	1.274	21.8
Gandul	*Cajanus Cajan (L.)*	Pigeon pea	[2]Escuela	73.7	0.48	2.9	1.035	1.36	31.7	127.0	1.51	.064	.330	.149	2.340	35.6

Gandul	*Cajanus Cajan (L.)* Millsp.	Pigeon pea	[2]Escuela	73.7	0.48	2.9	1.035	1.36	31.7	127.0	1.51	.064	.330	.149	2.340	35.6
Guajiniquil	*Inga sp.*	.....	[2]Tegucigalpa	64.2	0.18	1.3	0.680	.64	57.7	59.6	4.13	.284	.069	.022	.907	14.6
Haba	*Phaseolus limensis* Macf.	Lima bean	[2]Escuela	61.3	0.08	1.9	1.470	1.50	36.2	112.5	3.19	.427	.246	.110	1.511	3.2
Habichuela (a)	*Phaseolus vulgaris L.*	Green string bean	[2]Escuela	91.5	0.08	1.5	0.248	.62	53.0	33.0	1.27	.154	.055	.082	.488	27.2
Habichuela (b)	*Phaseolus vulgaris L.*	Green string bean	San Pedro Sula	87.6	0.35	1.5	0.539	.80	36.9	72.8	1.17	.016	.135	.081	.994	9.5
Paterna	*Inga sp.*	.....	Guinope	67.9	<0.10	1.0	1.065	.78	28.8	65.5	1.06	4.850	.148	.095	1.121	19.6
	Canavalia ensiformis (L.) DC.	Jack bean	[2]Escuela	78.5	0.50	3.3	1.109	.80	32.6	65.6	1.15	.030	.220	.104	2.000	31.6
	FRUITS														
	1. Pome fruits														
Icaco	*Chrysobalanus Icaco L.*	Cocoa plum, Icaco	Puerto Cortés	84.8	0.16	0.6	0.064	.99	42.8	18.5	0.87	.006	.034	.015	.356	5.6
Membrillo	*Cydonia oblonga* var. *mali-formis* (Mill.) Schneid.	Quince	Guinope	83.9	0.99	1.5	0.094	.35	5.7	15.3	0.98	.017	.036	.021	.186	6.8
Perote	*Malus sylvestris* Mill.	Apple	Guinope	83.1	<0.10	0.7	0.020	.25	6.4	9.2	1.08	.051	.024	.020	.206	6.8
	2. Stone fruits														
Ciruela tronadora	*Spondias Mombin L.*	Spanish plum	Danlí	72.8	<0.10	0.6	0.208	1.26	31.4	66.7	2.78	.071	.095	.050	.860	46.4
Coco	*Cocos nucifera L.*	Coconut meat	[2]Lancetilla	62.8	24.90	5.2	0.621	0.95	6.0	81.4	1.50	.004	.173	.086	.382	1.8
Coco, agua de	*Cocos nucifera L.*	Coconut milk	[2]Lancetilla	95.6	0.00	0.0	0.034	0.54	13.0	19.7	0.43	.004	.002	.000	.049	0.6
Coyol	*Acrocomia mexicana* Karw.	.....	[2]Escuela	47.9	18.55	4.5	0.457	....	....	....	....	.107	.114	.049	.600	28.2
Jambolana	*Eugenia Jambolana L.*	Jambolana plum	[2]Lancetilla	85.8	0.15	0.3	0.129	.32	8.3	16.2	1.62	.004	.008	.009	.290	5.7
Mango (a)	*Mangifera indica L.*	Mango	Guinope	81.6	0.35	1.2	0.033	.49	10.8	17.2	0.60	1.314	.072	.064	.577	129.0
Mango (b)	*Mangifera indica L.*	Mango	San Pedro Sula	88.8	0.45	0.6	0.094	.47	12.8	15.7	0.63	1.350	.030	.048	.432	60.2
Mango Zapato (c)	*Mangifera indica L.*	Mango	[2]Tegucigalpa	80.8	0.28	0.4	0.054	.42	7.9	12.5	0.56	.283	.046	.031	.416	146.6
Manzana rosa	*Eugenia Jambos L.*	Rose apple	[2]Guinope	85.1	0.18	1.9	0.085	.44	45.2	11.7	0.49	.123	.019	.028	.521	16.6
Pejibaye (a)	*Guilielma utilis* Oerst.	Pejibaye, red variety	[2]Lancetilla	36.4	8.17	0.8	0.633	1.12	14.4	55.2	1.16	2.760	.070	.140	1.945	22.9
Pejibaye (b)	*Guilielma utilis* Oerst.	Pejibaye, yellow variety	[2]Lancetilla	49.6	3.53	1.1	0.401	0.86	8.9	46.1	1.31	.835	.037	.099	1.493	41.4
	3. Berries														
Aberia	*Doryalis hebecarpa* (Gardn.) Warb.	Ceylon gooseberry	[2]Escuela	83.6	1.02	1.9	0.174	.63	12.6	26.8	1.41	.356	.017	.042	.261	91.7

TABLE 2 *(Concluded)*

Composition of Food Samples From Honduras

Name of food sampled			Place sample obtained [2]	Constituents measured and yields per 100 grams											
Spanish [1]	Scientific	English		Moisture	Ether extract	Crude fiber [3]	Nitrogen	Ash	Calcium	Phosphorus	Iron	Carotene	Thiamine	Riboflavin	Niacin	Ascorbic Acid-Total
				gm.	*gm.*	*gm.*	*gm.*	*gm.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Chachalaca	*Cordia dentata* Poir.	.....	[2]Chamelecón river at La Lima	86.7	0.38	0.1	0.199	1.19	6.9	40.9	1.32	.011	.001	.035	.494	21.0
Sarsil	*Ardisia escallonioides* Schlecht. and Cham.	.....	[2]Escuela	72.1	0.91	3.1	0.211	1.17	130.2	38.2	0.80	.012	.003	.013	1.554	10.4
Zarzamora	*Rubus hondurensis* Bail.	Blackberry	[2]Guinope	79.6	1.13	5.8	0.206	.60	28.6	40.1	1.53	.046	.046	.041	.357	66.3
	4. Citrus															
Lima o Limón (a)	*Citrus aurantifolia* (Christm.) Swingle	Lime	Guinope	91.4	0.17	0.5	0.112	.38	33.3	21.0	0.27	.022	.066	.023	.231	48.7
Lima o Limón (b)	*Citrus aurantifolia* (Christm.) Swingle	Lime	[2]Research Div. Garden	92.2	0.65	0.1	0.081	.33	15.3	17.4	1.71	.013	.009	.016	.080	36.7
Lima limón	*Citrus limetta* Risso	Sweet lime	San Pedro Sula	85.6	0.28	0.8	0.170	.43	33.6	23.0	0.79	.003	.031	.033	.233	44.3
Limón	*Citrus Limonia* Osbeck	Lemon	Guinope	94.3	0.99	0.4	0.123	.43	32.2	19.0	0.34	.031	.090	.025	.194	27.2
Limón ponderosa	*Citrus Limonia* Osbeck	Ponderosa lemon	[2]Escuela	93.7	1.35	0.0	0.039	.27	7.6	8.0	1.74	.003	.023	.007	.029	25.0
Mandarina (a)	*Citrus nobilis* var. *deliciosa* (Ten.) Swingle	Mandarin orange	[2]Tegucigalpa	89.6	0.32	0.3	0.121	.33	30.3	14.2	0.41	.046	.067	.022	.246	13.3
Mandarina (b)	*Citrus nobilis* var. *deliciosa* (Ten.) Swingle	Mandarin orange	La Lima	87.1	0.21	0.7	0.215	.41	27.3	19.8	0.38	.077	.085	.024	.269	14.0
Toronja (a)	*Citrus maxima* (Burm.) Merrill	Grapefruit	[2]Tegucigalpa	84.4	0.40	0.3	0.114	.52	29.0	23.5	0.63	.003	.043	.013	.261	44.6
Toronja (b)	*Citrus maxima* (Burm.) Merrill	Grapefruit	San Pedro Sula	89.9	0.38	0.1	0.079	.32	6.4	12.3	0.34	.003	.018	.010	.097	23.0
	5. Bananas															
Banano (a)	*Musa paradisiaca* var. *sapientum L.*	Banana	Yuscarán	59.7	0.20	0.4	0.139	.76	3.4	22.9	0.61	.064	.023	.033	.663	24.8
Banano (b)	*Musa paradisiaca* var. *sapientum L.*	Banana	[2]La Lima	74.8	0.09	0.3	0.172	.81	8.8	32.4	0.46	.148	.023	.037	.681	6.0
Banano (c)	*Musa paradisiaca* var. *sapientum L.*	Banana	[2]Research Div. Garden	58.9	0.69	0.3	0.170	1.07	3.5	27.5	0.87	.169	.053	.030	.610	16.0
Banano (d)	*Musa paradisiaca* var. *sapientum L.*	Banana	[2]Research Div. Garden	71.4	0.29	0.3	0.176	.99	5.7	27.9	0.41	.021	.025	.026	.670	8.7

Banano (d)	*Musa paradisiaca* var. *sapientum L.*	Banana	[2]Research Div. Garden	71.4	0.29	0.3	0.176	.99	5.7	27.9	0.41	.021	.025	.026	.670	8.7
Banano (e)	*Musa paradisiaca* var. *sapientum L.*	Banana	[2]Research Div. Garden	71.0	0.12	0.6	0.166	.84	12.3	22.6	0.76	.008	.032	.024	.930	8.6
Banano (f)	*Musa paradisiaca* var. *sapientum L.*	Banana	[2]Research Div. Garden	72.5	0.20	0.3	0.157	.89	11.2	24.8	1.48	.004	.025	.029	.770	6.8
Banano (g)	*Musa paradisiaca* var. *sapientum L.*	Banana	[2]Research Div. Garden	72.4	0.33	0.3	0.166	.93	8.9	24.8	0.46	.008	.031	.043	.800	7.1
Plátano (a)	*Musa paradisiaca L.*	Plantain	Zamorano	67.8	0.10	1.1	0.139	....	....	....	....	.071	.062	.037	.550	20.5
Plátano (b)	*Musa paradisiaca L.*	Plantain	Jicarito	70.1	1.17	0.4	0.187	.79	11.9	17.1	0.59	.619	.043	.027	.630	5.6
Plátano (c)	*Musa paradisiaca L.*	Plantain	[2]Research Div. Garden	69.2	0.28	0.4	0.171	1.01	6.5	48.1	0.49	.003	.048	.029	.630	18.5
Plátano (d)	*Musa paradisiaca L.*	Plantain	[2]Research Div. Garden	68.8	0.41	0.4	0.142	.94	6.6	47.3	0.80	.006	.042	.017	.560	16.7
Plátano macho (e)	*Musa paradisiaca L.*	Horse plantain	Danlí	58.8	0.20	0.3	0.151	.73	4.6	29.0	1.13	.732	.089	.038	.630	17.6
Plátano macho (f)	*Musa paradisiaca L.*	Horse plantain	[2]Research Div. Garden	60.0	0.64	0.3	0.191	1.11	2.8	31.6	1.94	.073	.075	.029	.640	23.8
Plátano macho (g)	*Musa paradisiaca L.*	Horse plantain	[2]Lancetilla	60.4	0.42	0.4	0.212	.76	6.0	28.6	4.89	.023	.057	.030	.790	20.3
	6. Other fruits															
Anona	*Anona squamosa L.*	Sweetsop	Yuscarán	69.8	0.12	2.5	0.309	1.34	44.7	55.3	1.02	.005	.100	.167	1.280	42.2
Caimito (a)	*Chrysophyllum Cainito L.*	Star apple	San Pedro Sula	85.7	0.64	1.2	0.130	.44	17.3	16.4	0.48	.015	.027	.018	1.053	9.1
Caimito (b)	*Chrysophyllum Cainito L.*	Star apple	[2]Lancetilla	79.8	1.92	0.7	0.139	.43	7.4	22.0	0.35	.018	.026	.013	.800	6.6
Carambola	*Averrhoa Carambola L.*	Carambola	[2]Lancetilla	89.0	0.71	0.8	0.128	.40	6.0	18.4	1.65	.003	.038	.022	.344	26.0
Cola	*Cola nitida (Vent.)* A. Chev.	.....	[2]Lancetilla	83.3	0.57	0.8	0.235	.24	3.1	16.9	1.35	.031	.011	.047	.667	9.8
Durio	*Durio zibethinus* Murr.	Durian	[2]Lancetilla	61.1	0.83	1.6	0.363	1.06	7.6	37.8	0.73	.018	.352	.202	.683	23.9
Higo	*Ficus glabrata* HBK.	Deer fig	[2]Near Zamorano	90.3	0.09	1.7	0.078	.71	61.9	7.0	2.30	.059	.027	.022	.185	tr.
Níspero	*Manilkara Zapotilla* (Jacq.) Gilly	Sapodilla	[2]Danlí	73.1	2.00	1.2	0.060	.48	28.9	20.8	1.54	.024	.001	.011	.247	9.2
Piña de agua (a)	*Ananas comosus (L.)* Merrill	Pineapple	Danlí	84.6	0.29	0.4	0.038	.28	16.4	10.7	1.05	.004	.081	.022	.123	85.2
Piña de azúcar (b)	*Ananas comosus (L.)* Merrill	Pineapple	Yuscarán	80.3	0.24	0.6	0.060	.36	10.6	5.2	0.65	.011	.080	.022	.160	165.2
Piña (c)	*Ananas comosus (L.)* Merrill	Pineapple	San Pedro Sula	77.8	0.08	0.5	0.101	.24	12.2	5.8	0.54	.004	.118	.036	.120	24.6
Zapote	*Pouteria mammosa (L.)* Cron.	Sapote	San Pedro Sula	65.7	0.18	3.1	0.240	.95	29.2	28.1	0.74	.175	.007	.010	1.913	9.2

[1] Names given are those used commonly in Honduras. [2] Samples collected at these places were taken from the plant; histories of other samples are uncertain.

tene (0.154 versus 0.016 mg.), and ascorbic acid (27.2 versus 9.5 mg.). It was lower in thiamine (0.055 versus 0.135 mg.) and niacin content (0.488 versus 0.994 mg.). None of these differences seems very significant.

Mango. The three samples represented fruits that varied considerably in appearance, flavor, and texture. They were very similar in composition except for differences in content of carotene (1.314, 1.350, and 0.283 mg.) and ascorbic acid (129.0, 60.2, and 146.6 mg.), due, no doubt, to varietal differences.

Pejibaye. Sample (a) of this food represented a red variety, Sample (b) a yellow variety. The most pronounced difference in composition was in carotene content (2.760 and 0.835 mg.). The moisture content of Sample (a) was lower than that of Sample (b), which allows for its higher content of several constituents. It will be noted that this food has a relatively high concentration of niacin.

Lima or *Limón.* Two samples of limes were analyzed. These differed mainly in their content of calcium (33.3 and 15.3 mg.), iron (0.27 and 1.71 mg.), and thiamine (0.066 and 0.009 mg.). Sample (a) comprised flesh and juice, while Sample (b) comprised juice only.

Mandarina. The two samples were collected at different places, but were very similar in composition.

Toronja. Both samples of grapefruit were prepared from fruits of poor quality. Sample (a) comprised flesh and juice, and Sample (b) juice only. The two samples showed significant differences in content of calcium (29.0 and 6.4 mg.), iron (0.63 and 0.34 mg.), thiamine (0.043 and 0.018 mg.), and ascorbic acid (44.6 and 23.0 mg.).

Banano. Seven varieties of banana were sampled. Samples (a) and (c) had a relatively low moisture content and a correspondingly higher content of ascorbic acid. Otherwise, the samples were remarkably uniform in composition.

Plátano. The outstanding differences in the composition of four samples of *plátanos* and the three samples of *plátanos machos* relate to content of iron, carotene, and ascorbic acid. All samples of *plátanos machos* had a higher content of iron (1.13, 1.94, and 4.89 mg.) than the *plátanos* (0.59, 0.49, and 0.80 mg.). Sample (a) of the *plátanos* and Sample (e) of the *plátanos machos* were highest in carotene content (0.619 and 0.732 mg., respectively). The ascorbic acid values varied from 23.8 mg. in Sample (f) to 5.6 mg. in Sample (b). This latter sample was purchased in the market and was of uncertain history.

Caimito. The two samples of star apple showed no important differences in composition. It should be noted that this fruit, like *pejibaye*, has a relatively high concentration of niacin.

Piña. Three varieties of pineapple were sampled. The most conspicuous differences in composition are in content of ascorbic acid (85.2, 165.2, and 24.6 mg.). The ascorbic acid content is undoubtedly a varietal characteristic here, as with other fruits.

Six of the samples analyzed were exceptionally rich in several nutrients. Paradoxically, these are all foods used to a limited extent, or not at all, even though they can be grown easily and are relatively cheap.

Chaya is an ornamental tree used for shade and found in limited numbers in Honduras. The leaf blade constitutes the edible portion. The sample was high in its content of calcium (333.6 mg.), iron (10.77 mg.), carotene (5.960 mg.), thiamine (0.222 mg.), riboflavin (0.526 mg.), niacin (2.000 mg.), and ascorbic acid (175.5 mg.).

Santa María is a small, weak tree growing in moist areas, especially where forests have been cut back, and is found commonly along the north coast of Honduras. The plant has an odor resembling sarsaparilla and the leaf blades are used occasionally as flavoring in soups or stews. The sample showed a high content of calcium (197.4 mg.), iron (6.54 mg.), carotene (1.910 mg.), riboflavin (0.204 mg.), and ascorbic acid (70.0 mg.).

Tampala is one of the pigweeds and grows readily with little or no cultivation. It may be used as a green, although the extent to which it is used in Honduras is not known. The sample was very rich in calcium (578.0 mg.) and contained relatively large amounts of iron (3.55 mg.), carotene (1.801 mg.), riboflavin (0.270 mg.), niacin (2.288 mg.), and ascorbic acid (86.4 mg.).

The extent to which leaves of the *yuca* (cassava) are used as food in Central America is not known. This plant is related to *chaya*, mentioned above. A toxic substance present in the leaf of the cassava hinders its use except after cooking. The sample gave a high yield of calcium (292.2 mg.), iron (8.08 mg.), carotene (6.520 mg.), thiamine (0.272 mg.), riboflavin (0.425 mg.), niacin (3.530 mg.), and ascorbic acid (320.5 mg.).

The last two samples listed under "Herbage Vegetables" are not identified with Spanish names. Both samples were taken from the Río Yeguare and are aquatic or sub-aquatic herbs. The first, *Heteranthera reniformis*, yielded a very high content of iron (18.47 mg.) and was relatively rich in carotene (1.673 mg.). The second, *Jussiaea repens*, yielded a high content of calcium (144.2 mg.), iron (8.03 mg.), carotene (3.940 mg.), and ascorbic acid (86.9 mg.).

A few other plants may be mentioned for their relatively high content of one or two nutrients. Among the herbage vegetables, *Rosa de Jamaica*, Sample (b), had a high content of calcium (103.0 mg.) and niacin (1.220 mg.). The stone fruit *Coyol* was very rich in fat (18.55 gm.). Of the berries, *Aberia* showed a good content of ascorbic acid (91.7 mg.) and *Sarsil* a high content of calcium (130.2 mg.) and niacin (1.554 mg.). Two other fruits showed a high content of niacin, *Anona* (1.280 mg.) and *Zapote* (1.913 mg.).

SUMMARY

Data are presented on the content of moisture, ether extract, crude fiber, nitrogen, ash, calcium, phosphorus, iron, carotene, thiamine, riboflavin, niacin, and ascorbic acid in 121 samples of food plants collected in Honduras from July, 1946 through April, 1947.

Several foods are represented by two or more samples collected at different times and in different places. Differences in the composition of the two or more samples of a food are discussed in relation to such factors as variety of the plant and method of sampling.

Attention is called to several foods with very high content of several constituents, but which appear to be used to a limited extent in the dietary of the people of Honduras.

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COMPARISON OF METHODS OF RECONSTITUTING MILK POWDER FOR THE PLATE COUNT, WITH AN ANALYSIS OF VARIANCE[1,2]

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Standard Methods for the Examination of Dairy Products (1941) is not specific in its recommendation of methods for reconstituting milk powder for the plate count. It permits various options intended to assist in putting the powder into homogeneous dispersion; the water may be warmed to from 43 to 49°C.(109 to 120°F.), or for extremely insoluble samples a solution of N/10 lithium hydroxide may be used instead of water.

Recent reports indicate that the option chosen may have a distinct influence upon the resulting count. Hiscox (1945) found that counts were higher if the powder was reconstituted in a blank previously warmed to about 50°C.(45 to 55°C.) than if the blank were at room temperature when the powder was added. Her findings have been confirmed by Higginbottom (1945), Findlay *et al.* (1946), Speck and Meyers (1946), and White (1947). Prickett and Miller (1932, 1933) found that N/10 lithium hydroxide blanks exerted no bactericidal effect when used for the reconstitution of powder, but according to Higginbottom (1944), Garrison (1946), Speck and Meyers (1946), and White (1947), counts were lowered when the powder was reconstituted in lithium hydroxide solutions.

According to Higginbottom (1944), alkali solutions more dilute than 10th normal, when used as dispersing agents for insoluble powder, offered no advantage over water. Solutions of alkaline salts, such as citrates and phosphates, have been suggested for this purpose, Speck and Meyers (1946), Garrison (1946), but the published data are too meager to establish their advantages or disadvantages.

In the present work several methods of reconstituting milk powders have been compared on the basis of counts obtained with them. The analysis of variance has been used to determine the significance of the differences in results obtained with the different treatments. The analysis of variance is a statistical method which separates and isolates the different components of variance, so that their significance can be established with fewer determinations than would otherwise be required.

METHODS

Only spray-dried whole milk powders were used in these experiments. For plating, they were first reconstituted to a concentration of 12.5 per

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cent milk solids, which is about average for whole milk, and further dilutions made in water as required. Quantities used were 10 grams of powder in 70 ml. of liquid. Blanks used for reconstitution were tempered either in air at room temperature or in a water bath at a higher temperature, according to the plan of the experiment. Blanks were prepared in four-ounce screw-capped, wide-mouth jars which permitted rapid introduction of the powder sample.

The powder was weighed aseptically into a sterile, flexible aluminum dish (milk bottle cap) which, by means of slight pressure on the sides, could be shaped as a scoop for rapid transfer of the powder into the jar containing the blank. The mixture was immediately shaken for 30 seconds by a procedure consisting of 100 to 120 one-foot excursions. This procedure was found by Cone and Ashworth (1947) to give complete wetting of most spray-dried whole milk powders. Hiscox (1945) also employed a 30-second shaking procedure, but with only 50 one-foot excursions. Except when otherwise specified, the reconstituted sample was held for five minutes at the temperature at which the blank was tempered; the sample was then diluted in water at room temperature and plated in duplicate. The medium used was tryptone glucose meat-extract milk agar (standard milk agar). The plates were incubated at 30°C.(86°F.) for four days.

On the data from each experiment an analysis of variance was made according to procedures outlined by Snedecor (1946). This required reporting the count from each individual plate separately; to simplify the computation, all counts were converted to two-place logarithms. Whenever the analysis of variance showed there was a significant difference between treatments, the "95 per cent fiducial limits" were calculated to indicate which treatments were significantly different and which were not. Whenever the fiducial limits of two treatment means do not overlap, one can conclude with confidence that differences between the treatments were significant and not due merely to experimental error.

RESULTS

Effect of Temperature of Water: In a comparison of reconstitution temperatures, counts were made on 15 lots of powder, each reconstituted at room temperature (20 to 25°C.), 45, 50, and 55°C. After reconstitution the samples were held for five minutes at their respective temperatures before they were diluted and plated. Results, together with the analysis of variance, are given (Table 1).

The analysis of variance shows that the difference between treatments is significant beyond the one per cent confidence level. An inspection of the treatment shows that lowest results were obtained with reconstitution at room temperature, while reconstitution at 50°C. gave results slightly higher than those obtained with reconstitution at either 45 or 55°C. The calculated fiducial limits of the treatment means are as follows:

Room temperature	2.927-3.121
45°C.	3.144-3.238
50°C.	3.221-3.315
55°C.	3.191-3.285

TABLE 1

Effect of Temperature of Water of Reconstitution on Bacterial Plate Count of Milk Powder

Sample No.	Temperature of water of reconstitution			
	Room temp.	45°C.	50°C.	55°C.
	count[1]	*count*	*count*	*count*
56	4.11	4.17	4.23	4.08
	4.12	4.19	4.26	4.05
63	4.77	4.90	4.86	4.92
	4.76	4.90	4.85	4.83
64	3.51	3.65	3.76	3.57
	3.64	3.52	3.66	3.43
65	3.51	3.65	3.72	3.61
	3.48	3.67	3.51	3.57
115	2.85	2.90	2.94	2.81
	2.83	2.79	2.89	2.88
116	3.30	3.46	3.75	3.64
	3.48	3.57	3.81	3.78
118	2.45	2.48	2.59	2.65
	2.30	2.60	2.65	2.65
119	2.61	2.63	2.84	2.88
	2.40	2.84	2.90	2.86
128	2.86	2.93	3.05	2.90
	2.79	2.92	3.03	2.94
129	2.61	2.64	2.71	2.73
	2.71	2.72	2.67	2.70
130	2.26	2.71	2.76	2.94
	2.30	2.59	2.87	2.90
131	2.58	2.72	2.66	2.72
	2.64	2.72	2.58	2.64
133	2.79	2.86	3.00	2.97
	2.82	2.90	3.11	2.92
134	2.75	2.84	2.99	2.94
	2.81	3.01	2.92	2.91
136	3.05	3.12	3.23	3.33
	3.12	3.13	3.25	3.39
Mean	3.074	3.191	3.268	3.238

[1] Count in logarithms.

Analysis of Variance

Source of variation	d.f.	Sum of squares	Mean square
Total	119	48.9668	.....
Powders	14	47.3932	.....
Counts	60	0.2388	0.0040
Treatments	3	0.6583	0.2194[2]
Powders × treatments	42	0.6765	0.0161

[2] Significant beyond the one per cent confidence level.

It appears, therefore, that there was no significant difference among the results for the three higher temperatures of reconstitution. However, reconstitution at room temperature consistently gave results distinctly lower than those obtained at the higher temperatures. As a result of this experiment, reconstitution in water at 50°C. was selected as a standord with which to compare all other reconstitution treatments in the following experiments.

Effect of Time of Holding the Reconstituted Sample at 50°C.: Higginbottom (1945) adopted the procedure of returning the reconstituted sample to the 50°C. bath in which the Ringer's solution was tempered and holding it at that temperature for 15 minutes before proceeding with the plating. At times such a long holding period could become cumbersome; therefore, an experiment was performed to determine the effect of holding the sample for shorter periods.

Thirteen powders were used in this experiment. Distilled water at 50°C. was used as the reconstituting fluid. Four samples of each lot of powder were reconstituted by the usual 30-second shaking procedure. One sample was diluted immediately, and plated. The other three were held at 50°C. for five, 10, and 15 minutes, respectively, before they were plated. Results obtained by the different treatments, and the analysis of variance, are shown (Table 2).

The differences in counts resulting from the different treatments were shown not to be significant, by the analysis of variance. However, for uniformity and convenience, the five-minute holding period was adopted as standard procedure in subsequent experiments (and will be that shown by our data, unless otherwise stated).

Effect of Mechanical Shaking: Higginbottom (1945) attributed the increase in count resulting from reconstitution of powder at 50°C., over that at room temperature, to better dispersion of the powder and bacterial clumps. Presumably, prolonged mechanical shaking, especially with beads, would tend to accomplish the same results.

In an experiment designed to test the effect on plate counts caused by prolonged mechanical shaking during reconstitution, eight lots of powder were used. Seven samples of each lot of powder were weighed out. As a standard of comparison, one sample was reconstituted with water at 50°C., held at 50°C. for five minutes, then plated. The other samples were reconstituted with water at room temperature by manual shaking, then placed in a mechanical shaker. Three of the jars contained a teaspoonful of beads each, while three contained no beads. After intervals of two, five, and 15 minutes of shaking, one jar with beads and one without was removed from the shaker and samples plated immediately. Plate counts obtained and analysis of variance are shown (Table 3).

The analysis of variance revealed that differences in count resulting from differences in treatment were significant beyond the one per cent confidence level. The fiducial limits were found to be as follows:

Standard (water at 50°C.)	3.560-3.466
Two minutes without beads	3.360-3.266
Five minutes without beads	3.363-3.351
Fifteen minutes without beads	3.445-3.351
Two minutes with beads	3.375-3.281
Five minutes with beads	3.450-3.356
Fifteen minutes with beads	3.409-3.315

TABLE 2

Effect of Time of Holding Reconstituted Sample at 50°C.

Sample No.	Minutes held at 50°C. after reconstitution			
	0	5	10	15
	count[1]	*count*	*count*	*count*
47	2.48	2.32	2.43	2.46
	2.51	2.43	2.40	2.43
48	2.57	2.48	2.53	2.43
	2.49	2.45	2.38	2.46
115	2.83	2.94	2.83	2.86
	2.83	2.89	2.89	2.82
116	2.76	2.75	2.63	2.59
	2.67	2.81	2.77	2.59
117	2.53	2.66	2.64	2.53
	2.42	2.64	2.66	2.64
118	2.70	2.59	2.72	2.61
	2.66	2.65	2.71	2.69
119	2.89	2.84	2.89	2.94
	2.88	2.90	2.86	2.90
120	2.34	2.51	2.43	2.18
	2.15	2.42	2.18	2.32
128	3.04	3.05	3.15	3.13
	3.08	3.03	3.15	3.09
129	2.76	2.71	2.67	2.77
	2.67	2.67	2.65	2.74
130	2.86	2.76	2.90	2.88
	2.80	2.87	2.93	2.94
133	3.00	3.00	2.99	3.07
	3.02	3.11	2.99	3.09
134	2.66	2.99	3.05	2.96
	2.88	2.92	3.09	3.03
Mean	2.711	2.746	2.751	2.736

[1] Count in logarithms.

Analysis of Variance

Source of variation	d.f.	Sum of squares	Mean square
Total	103	5.9603	.....
Powders	12	5.4434	.....
Counts	52	0.1806	0.0035
Treatments	3	0.0247	0.0082
Powders × treatments	36	0.3116	0.0087

It will be seen from a study of these fiducial limits that none of the shaking procedures produced results as high as those obtained with reconstitution of 50°C. The closest approach was with the procedure of shaking the reconstituted milk with beads for five minutes. Much of the superiority shown by this treatment over the other shaking procedures may be attributed to the results of one sample, Powder No. 119. The reason for the unusually high counts in this sample is not apparent.

Although there were some apparent differences in results between the different shaking procedures, the fiducial limits overlapped in all instances. Therefore, on the basis of the small number of samples employed here, one could not say the differences between various shaking procedures were significant.

TABLE 3

Effect of Prolonged Mechanical Shaking in Water at Room Temperature as Compared to Reconstitution in Water at 50°C.

Sample No.	Reconst. in water at 50°C.	Minutes subjected to mechanical shaking					
		Without beads			With beads		
		2	5	15	2	5	15
	count[1]	*count*	*count*	*count*	*count*	*count*	*count*
56N	4.52	4.41	4.41	4.45	4.43	4.44	4.48
	4.53	4.40	4.41	4.48	4.45	4.43	4.44
63	4.86	4.69	4.79	4.87	4.69	4.87	4.76
	4.85	4.69	4.85	4.83	4.81	4.70	4.86
64	3.76	3.62	3.32	3.61	3.52	3.52	3.61
	3.66	3.56	3.67	3.72	3.52	3.63	3.61
65N	3.82	3.60	3.66	3.67	3.76	3.70	3.63
	3.85	3.75	3.72	3.71	3.79	3.75	3.71
118	2.64	2.38	2.18	2.52	2.53	2.34	2.40
	2.75	2.30	2.49	2.52	2.30	2.51	2.46
119	2.81	2.54	2.51	2.64	2.51	3.00	2.59
	2.76	2.43	2.70	2.60	2.53	2.83	2.59
131	2.66	2.51	2.42	2.53	2.48	2.42	2.53
	2.58	2.45	2.42	2.42	2.48	2.53	2.48
133	3.11	2.85	2.76	2.90	2.62	2.85	2.89
	3.03	2.83	2.75	2.91	2.82	2.91	2.76
Mean	3.513	3.313	3.316	3.398	3.328	3.403	3.362

[1] Count in logarithms.

Analysis of Variance

Source of variation	d.f.	Sum of squares	Mean square
Total	111	81.9015	.....
Powders	7	80.7380	.....
Counts	56	0.3231	0.0058
Treatments	6	0.4753	0.0792[2]
Powders × treatments	42	0.3651	0.0087

[2] Significant beyond the one per cent confidence level.

Effect of Reconstituting With Alkaline Solutions at Room Temperature: While alkaline solutions might be expected to aid in the dispersion of the powder and the bacterial clumps, there is little to be gained if, at the same time, they are sufficiently toxic to reduce the count. In an experiment designed to test the net effect of alkaline solutions at room temperature as media for reconstituting powder for plate counts, N/10 LiOH, N/60 NaOH, 1.25 per cent disodium phosphate ($Na_2HPO_4 \cdot 12H_2O$), and 1.25 per cent sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$) solutions were compared with water at room temperature and with water at 50°C. Eight lots of powder were used. Results and the analysis of variance are shown (Table 4).

The analysis of variance shows that differences between treatments are highly significant, and inspection of the treatment means reveals results

TABLE 4

Effect of Various Alkaline Solutions at Room Temperature as Compared With Water at Room Temperature and at 50°C.

Sample No.	Reconst. in water at 50°C.	Reconstitution liquid at room temp.				
		Water	N/10 LiOH	N/60 NaOH	1.25 pct. PO_4	1.25 pct. citrate
	count[1]	*count*	*count*	*count*	*count*	*count*
56	4.23	4.11	3.79	3.92	3.90	3.86
	4.26	4.12	3.77	3.92	4.01	3.93
63	4.86	4.77	4.32	4.75	4.61	4.63
	4.85	4.76	4.52	4.66	4.71	4.71
64	3.76	3.51	3.28	3.76	3.60	3.59
	3.66	3.64	3.43	3.64	3.42	3.71
65	3.72	3.51	3.34	3.54	3.46	3.57
	3.51	3.48	3.36	3.59	3.54	3.57
119	2.81	2.61	2.56	2.58	2.67	2.74
	2.76	2.40	2.51	2.73	2.67	2.63
130	2.81	2.26	2.57	2.70	2.48	2.43
	2.73	2.30	2.40	2.62	2.48	2.43
131	2.66	2.58	2.00	2.52	2.45	2.52
	2.58	2.64	2.32	2.43	2.42	2.52
133	3.11	2.79	2.54	2.90	2.85	3.02
	3.03	2.82	2.83	2.67	2.85	2.99
Mean	3.458	3.269	3.097	3.309	3.258	3.303

[1] Count in logarithms.

Analysis of Variance

Source of variation	d.f.	Sum of squares	Mean square
Total	95	55.0423	.....
Powders	7	53.0650	.....
Counts	48	0.4137	0.0086
Treatments	5	1.0750	0.2150[2]
Powders × treatments	35	0.4885	0.0140

[2] Significant beyond the one per cent confidence level.

for the lithium hydroxide solution markedly lower than for any of the other treatments. Calculating the fiducial limits of the treatment means, one finds:

Water at 50°C	3.518-3.398
Water at 22 to 25°C	3.329-3.209
N/10 LiOH	3.157-3.037
N/60 NaOH	3.369-3.249
1.25 pct. PO_4	3.318-3.198
1.25 pct. citrate	3.363-3.243

Again, water at 50°C. was without doubt a better reconstituting liquid than were any of the other treatments. At room temperature, water, N/60 NaOH, 1.25 per cent disodium phosphate, and 1.25 per cent sodium citrate solutions differed but little in net results obtained. Also, the dilute alkali and the mildly alkaline salt solutions showed little superiority to water at room temperature, as dispersing agents for insoluble powder.

The lithium hydroxide solution was definitely inferior to all other liquids used in this experiment. Contrary to expected results, dispersion of the powders in the lithium hydroxide was very poor, although all samples were spray-dried whole milk powders with good or average solubility indexes. The powder tended to remain in tiny lumps that did not completely disperse even after two or three minutes of manual shaking. Failure of the powder to disperse completely may have accounted, at least in part, for the lowered counts.

Effect of Alkaline Solutions at 50°C.: Since the use of mildly alkaline solutions as reconstituting liquids at room temperature gave net results differing but little from those of water at room temperature, it was felt they might be used with safety at 50°C. for those powders that might not disperse well in distilled water. Garrison (1946) had found sodium citrate solution at 45°C. quite satisfactory.

The same alkaline solutions that were used at room temperature, including N/10 LiOH, were used also in this experiment. In addition, a 0.5 per cent sodium citrate solution, used by Speck and Meyers (1946), was included; and neutral quarter-strength Ringer's solution, generally favored as a reconstituting medium in laboratories of Great Britain, was included to furnish a comparison between it and water at 50°C. Fifteen lots of powder were used in this experiment. Results and the analysis of variance are given (Table 5).

Again, results with N/10 LiOH were the lowest. Furthermore, the solution was objectionable because of excessive foaming. In addition, dispersion of the powder in lithium hydroxide was little better at this temperature than at room temperature.

The analysis of variance reveals differences between treatments that are highly significant. Fiducial limits of the treatment means were calculated to be as follows:

Water	3.308-3.228
Ringer's solution	3.293-3.213
0.5 pct. citrate	3.255-3.175
1.25 pct. citrate	3.232-3.152
1.25 pct. PO_4	3.246-3.160
N/10 LiOH	3.016-2.936
N/60 NaOH	3.202-3.120

TABLE 5

Effect of Alkaline Solutions as Compared With Ringer's Solution and With Water, All at 50°C.

Sample No.	Liquid used for reconstitution						
	Water	Ringer's solution	0.5 pct. citrate	1.25 pct. citrate	1.25 pct. PO_4	N/10 LiOH	N/60 NaOH
	count[1]	*count*	*count*	*count*	*count*	*count*	*count*
56	4.23	4.31	4.21	4.21	4.15	3.83	3.94
	4.26	4.34	4.26	4.25	4.17	3.86	4.04
63	4.86	4.74	4.75	4.85	4.73	4.59	4.85
	4.85	4.86	4.82	4.86	4.68	4.52	4.81
64	3.76	3.61	3.49	3.56	3.42	3.36	3.53
	3.66	3.61	3.49	3.34	3.60	3.42	3.68
65	3.72	3.73	3.48	3.49	3.52	3.49	3.51
	3.51	3.64	3.48	3.54	3.40	3.46	3.60
115	2.94	2.83	2.90	2.86	2.81	2.76	2.78
	2.89	2.89	2.82	2.81	2.81	2.62	2.75
116	3.75	3.54	3.56	3.25	3.43	3.40	3.60
	3.81	3.59	3.48	3.51	3.46	3.40	3.51
118	2.59	2.70	2.69	2.65	2.83	2.38	2.54
	2.65	2.70	2.67	2.65	2.72	2.42	2.63
119	2.84	2.84	2.81	2.66	2.72	2.36	2.64
	2.90	2.85	2.83	2.73	2.84	2.28	2.68
128	3.05	3.02	2.97	2.92	2.99	2.63	2.92
	3.03	3.00	3.04	3.00	2.98	2.69	2.94
129	2.71	2.65	2.70	2.91	2.86	2.65	2.69
	2.67	2.76	2.79	2.74	2.71	2.67	2.74
130	2.76	2.83	2.86	2.98	2.91	2.62	2.71
	2.87	2.81	2.83	2.76	2.91	2.58	2.67
131	2.66	2.78	2.61	2.68	2.59	2.54	2.62
	2.58	2.76	2.57	2.66	2.52	2.40	2.58
133	3.00	2.93	3.03	3.06	3.02	2.58	2.78
	3.11	3.03	3.02	2.90	3.03	2.68	2.95
134	2.99	2.97	3.00	2.68	3.07	2.57	2.96
	2.92	3.04	3.03	3.08	3.10	2.59	2.91
136	3.23	3.14	3.16	3.09	3.09	2.93	3.17
	3.25	3.10	3.11	3.08	3.11	2.99	3.14
Mean	3.268	3.253	3.215	3.192	3.206	2.976	3.162

[1] Count in logarithms.

Analysis of Variance

Source of variation	d.f.	Sum of squares	Mean square
Total	209	78.0660	.....
Powders	14	74.8661	.....
Counts	105	0.4427	0.0042
Treatments	6	1.7187	0.2865[2]
Powders × treatments	84	1.0385	0.0124

[2] Significant beyond the one per cent confidence level.

Results for N/60 NaOH were significantly lower than those for water or Ringer's solution. Apparently, the higher pH of the alkali solution acted in some way to oppose the heat effects evident in the case of water and Ringer's solution. However, the lower results obtained with N/60 NaOH are not evidence of true toxicity, since these results are distinctly higher than those shown for the same powders (Table 1) for reconstitution with water at room temperature.

To a smaller degree, the alkaline salt solutions also appeared to give lower results at 50°C. than did water or Ringer's solution. As shown by the overlapping of fiducial limits, statistical significance of these differences was not established by the few samples employed in this experiment; however, the trend toward lower counts resulting from reconstitution with the salt solutions was fairly consistent, and the higher the salt concentration the more pronounced was that trend. It is possible that a larger number of trials would show that the differences were significant.

DISCUSSION

In agreement with recent reports, our results show that reconstitution of milk powder in water at 50°C. results in a higher plate count than would be obtained by reconstitution in water at room temperature. Higginbottom (1945) attributed the higher counts to better dispersion of the bacterial clumps at the higher temperature. In our studies neither prolonged mechanical shaking with beads, nor use of alkaline solutions at room temperature, gave any significant increase in counts over those obtained with the regular reconstitution procedure with water at room temperature. Working with dried milk cultures of *Lactobacillus bulgaricus*, Speck and Meyers (1946) found by microscopic examination that dispersion of the bacterial clumps was just as complete when the powder was reconstituted at room temperature as when water at 50°C. was used. They believed, as did Hiscox (1945), that heat stimulation of the bacterial cells at the moment of hydration resulted in a greater number of dormant cells becoming active. Warming the solution to 50°C. after reconstitution at room temperature produced no such stimulation.

The mechanism of the stimulation has not been explained. From data presented here it is clear that the higher pH of N/60 NaOH in some way opposes the stimulating action of the heat. The data indicate also that the addition of salts to the water may decrease the stimulating effect of reconstitution at 50°C. Apparently, the presence of excess electrolytes interferes with the optimal hydration of the cell constituents at the higher temperatures.

Contrary to the findings of Prickett and Miller (1933), N/10 LiOH appears to exert a toxic effect both at room temperature and at 50°C. Ordinarily, the lithium hydroxide solution would not be used with spray-dried powders, since they usually are sufficiently soluble to be satisfactorily reconstituted with water. Higginbottom (1944) preferred water to the alkali solution, even for roller-dried powders. In view of the toxic effect of N/10 LiOH, as shown not only by our results but also by the results of others, it is doubtful that it should ever be used for the reconstitution of milk powder for the plate count, except when acid products are being examined.

Howat and Wright (1933) have shown that reconstitution at 50°C. gives definitely more complete dispersion of insoluble powders than does reconstitution at room temperature. Because reconstitution at 50°C. also gives higher counts, it appears timely to review the reconstitution procedures now recommended by *Standard Methods for the Examination of Dairy Products (1941)*, also the procedure recommended by the American Dry Milk Institute (1947), with a view to obtaining more satisfactory standardization of the procedures.

SUMMARY AND CONCLUSIONS

Reconstitution of spray-dried whole milk powder in water at 45, 50, or 55°C. resulted in much higher plate counts than when water at room temperature (22 to 25°C.) was used. Results obtained by reconstitution at 50°C. appeared to be slightly higher than those for 45 and 55°C., but the differences lacked statistical significance. Reconstituting at 50°C. and holding the reconstituted sample at 50°C. for five or 10 minutes appeared to be slightly better than plating immediately without holding, or holding at 50°C. for 15 minutes, but again the differences lacked statistical significance.

Reconstituting in water at room temperature and subjecting the reconstituted sample to mechanical shaking, with and without beads, for periods of two, five, and 15 minutes gave no significant increase in count over that obtained without the mechanical shaking. Efforts to increase dispersion of the bacteria by the use of alkaline water blanks, to obtain better powder dispersions, also resulted in no significant increase in counts. The use of 10th-normal lithium hydroxide significantly lowered the counts.

At 50°C. results obtained with water and with quarter-strength Ringer's solution were approximately the same. Solutions of sodium citrate (0.5 and 1.25 per cent) and of disodium phosphate (1.25 per cent) appeared to interfere slightly with the heat activation of the cells, and 60th-normal sodium hydroxide markedly interfered with heat activation. None of these solutions at room temperature exhibited any toxicity. This suggests that the phenomenon of heat activation may be associated with rapid hydration of some cell constituent, which process is depressed by the excess electrolytes.

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ASCORBIC ACID AND CAROTENE CONTENT OF PLANTAIN

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Plantain is used extensively in tropical countries, serving as a valuable food staple because, in part, it takes the place of grain and root vegetables. The fruits are generally cut while green and firm, and they are baked or boiled, either whole or in slices. They can also be dried and ground to a flour for use in making cakes, bread, and other bakery products. They belong to the same family as the banana, but are larger, coarser, and less sweet. Occasionally they are purchasable as "cooking bananas" in the markets of our larger cities.

Not much is known about the chemical composition and nutritive value of plantain. The water content is given as 63.8 per cent,[2] total carbohydrate, 30.6 per cent, 18.9 per cent total sugars, 11.7 per cent starch, and only 1.2 per cent protein. Fat, pectin, and ash content are low, 0.3, 0.4, and 0.85 per cent, respectively. Vitamin figures available for powdered dehydrated plantain are thiamine, 1.8; riboflavin, 2.8; and niacin, 15 micrograms per gram, dry basis. The content of vitamin C, determined by dye titration and potentiometric measurements of an extract of the pulp, was found to be 15.6 mg. per 100 grams of pulp for the green (Color 1) horse plantain, which increased to 21 mg. at Color 7. Deep frying caused some destruction (40 per cent), boiling much more, depending on the stage of ripeness.

These figures are somewhat irregular and are considerably higher than those given for bananas (10 to 13 mg.); at the time they were obtained, the presence and biological equivalence of dehydroascorbic acid were not adequately recognized. For these reasons, and because of the extensive use of plantain as human food and its possible utilization as an animal foodstuff, plantain was assayed for its content of ascorbic and dehydroascorbic acids, and for carotene.

Through the courtesy of United Fruit Company and of Mr. G. L. Poland of the Research Department, several green bunches of horse plantain were supplied us. These were allowed to ripen at 20°C.(68°F.), and portions were analyzed during the progress of the color change from 1 to 8, before and after cooking. Changes in carotene content were also measured.

The method used for the determination of ascorbic and dehydroascorbic acids was that of Roe and Oesterling (1944), except that bromine was used for the oxidation of ascorbic acid, Association of Vitamin Chemists, Inc.

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[2] Unpublished data, United Fruit Company.

(1947), Mills and Roe (1947). Results with bromine duplicated those obtained with norit.

Methods employed were as follows: Selected fingers were removed from a bunch in storage at 20°C. and any foreign material washed or wiped off. Two cuts were made crosswise of the finger through peel and pulp, the section rapidly peeled, and an approximately five-gram portion accurately weighed and placed in the acid medium for extraction. In taking a duplicate sample, about half an inch of the cut plantain was discarded. Extraction was carried on in a Waring blendor for from five to 10 minutes with 100 c.c. of a solution containing five per cent of metaphosphoric acid and one per cent of thiourea; the homogeneous extract was filtered by suction.

For the determination of dehydroascorbic acid, four c.c. of the filtrate were placed in each of two colorimeter tubes. To each was added one c.c. of two per cent 2,4-dinitrophenylhydrazine in approximately nine normal sulphuric acid, and they were held at 37°C.(98.6°F.) for three hours. A third tube, serving as a blank and containing water and one c.c. of the reagent, was also prepared. After the three tubes were cooled in an ice bath, five c.c. of 85 per cent sulfuric acid were added slowly (in not less than one minute) from a burette to each tube while in the ice bath. Rise in temperature can char, and produce positive errors. Following thorough shaking and cooling in the bath, the tubes were removed and after thirty minutes read in a photoelectric colorimeter with a 540-mμ. filter. For avoiding interfering colors from substances in plant extracts, this filter has since been found more satisfactory than the 520-mμ., Mills and Roe (1947).

The necessary calibration curve was made with standard dehydroascorbic acid solutions prepared from ascorbic acid; 10 mg. were dissolved in 100 c.c. of five per cent metaphosphoric acid solution and two drops of bromine added. After gentle shaking until the solution was slightly yellow, the excess of bromine was decanted, and air bubbled through the solution until it was colorless. Standards of appropriate concentration were made by diluting with a solution containing five per cent metaphosphoric acid and one per cent thiourea. The color was developed in the same manner as described above.

For the determination of total ascorbic acid, the original blendor filtrate was treated with bromine as just described, thiourea added, and the color developed as before. This reading, minus that for dehydroascorbic acid, represents ascorbic acid.

For the determination of carotene, the depth of color of an extract was compared with that of a standard solution of carotene. Since xanthophyll is the only other pigment present, it can be extracted with 85 per cent phosphoric acid as a blue compound, Haagen-Smit *et al.* (1943), leaving the unchanged carotene in the organic solvent.

The procedure was as follows: A 10-gram sample of plantain tissue was extracted with a foaming solvent, Moore and Ely (1941), comprising 150 c.c. of 95 per cent alcohol and 75 c.c. of petroleum ether (skelly-solve B) in the blendor for from five to 10 minutes. If the mixture did not foam, more alcohol was added, but a volume larger than 250 c.c. was found to be

impractical. The contents of the blendor were quantitatively transferred to a separatory funnel and the blendor rinsed successively with approximately 50 c.c. of petroleum ether, 25 c.c. of alcohol, and 150 c.c. of water containing five per cent sodium sulfate. This latter solution and the proportions of the three solvents helped to prevent formation of troublesome emulsions. The water alcohol layer was drawn into a second separatory funnel, any solid material packed at the bottom being pushed through the opened stopcock with a wire.

The water solution was washed three times with 30 c.c. of petroleum ether; the combined washings and the original petroleum ether extract were washed once with 50 c.c. of water and the separated petroleum ether concentrated to 25 c.c. To this solution (which need not be dry) four c.c. of 85 per cent phosphoric acid were added, then shaken in a 25-c.c. glass-stoppered graduate. With a 470-mμ. filter the color in the ether phase was compared with that of standard solutions of carotene (90 per cent β, 10 per cent α) and the amount of carotene determined from a calibration curve previously prepared.

TABLE 1

Changes in Amounts of Ascorbic Acid, Dehydroascorbic Acid, and Carotene in Plantain at Different Stages in Ripening[1]

Color number	Ascorbic + dehydro-ascorbic acid	Dehydro-ascorbic acid	Ascorbic acid	Caro-tene
1	18.0	3.1	14.9	.25
2	17.7	3.0	14.7	.30
3	17.5	2.7	14.8	.32
4	18.1	3.0	15.1	.39
5	14.8	4.9	9.9	.35
6	16.2	4.9	11.3	.24
7	13.6	10.6	3.0	.29
8	12.8	11.1	1.7	.34

[1] The average values are expressed as mg. per 100 grams of fresh pulp.

RESULTS AND DISCUSSION

Data on the ascorbic acid content are presented as averages (Table 1). With the exception of the figures for full yellow (Color 5) they indicate that during the ripening, up to Stage 5, there is little change in the amounts of either form of ascorbic acid. After Stage 6 the ascorbic acid content falls rapidly to a very small figure in the black fruit, with an increase in the dehydro form which does not quite correspond, such that there is an over-all decrease of 25 to 30 per cent in total ascorbic acid in the overripe fruit. Whether the high figure for dehydroascorbic acid represents physiologically active vitamin C, or includes inactive decomposition products such as di-keto-l-gulonic acid, would have to be determined by dye titration, bio-assay, or by a recently reported method for this acid, Roe *et al.* (1948). In any case, since plantain is not commonly used beyond Stages 5 or 6, it may be said that 15 to 18 mg. of total ascorbic acid are provided by 100 gm. of the pulp.

The ratio of ascorbic to dehydroascorbic acid in plantain is much higher than in the common vegetables. Goldblith and Harris (1948). In garden-

fresh vegetables, 90 per cent of the total ascorbic acid is in the reduced form; in green plantain only 20 to 30 per cent. No comparable figures seem to be available for the banana; the "ascorbic acid" content, presumably total, is reported as 10 to 11 mg. per 100 grams, Harris and Poland (1939). Another figure, which does not include dehydroascorbic acid, Thornton (1946), is 13 mg. The figure given in the Nutritional Charts of H. J. Heinz Company is 28, the distribution being unspecified. Expecting this latter figure, plantain is as well supplied with vitamin C as banana, perhaps a little better.

Since bananas are usually eaten raw and plantains never, the question of destruction during cooking required consideration. Results of a few observations which show that the partially ripe fruit, when baked or put in boiling water for 30 to 45 minutes, in the peel, lost less than 10 per cent of its vitamin C content (less by baking than by boiling) are shown (Table 2). The fully ripe fruit suffered much greater loss, 50 to 75 per cent, perhaps as the result of the diminished protection afforded by the softer peel, which splits easily during heating.

TABLE 2

Effect of Cooking on the Ascorbic and Dehydroascorbic Acid Content of Plantain[1]

Cooking treatment	Ascorbic + dehydro-ascorbic acid	Dehydro-ascorbic acid	Ascorbic acid
Partially ripe			
Control	17.8	2.9	14.9
Baked	17.0	2.7	14.3
Boiled	16.3	2.6	13.7
Fully ripe			
Control	15.5	4.9	10.6
Baked	7.2	2.1	5.1
Boiled	5.2	1.4	3.8

[1] The values are expressed as mg. per 100 grams of fresh pulp.

The carotene content of plaintain (Table 1, last column) remained remarkably constant during the ripening period. On the unjustified assumption that it is all β-carotene, the vitamin A content of plantain would be approximately 500 I. U. per 100 gm., slightly in excess of that of the banana.

SUMMARY

Plantain contains 15 to 18 mg. of total ascorbic acid per 100 grams of pulp. Contrary to the relation existing in common garden vegetables and in lemon juice, where 90 per cent of the total ascorbic acid is in the reduced form, in green plantain dehydroascorbic acid is only 20 to 30 per cent of the total.

As the fruit ripens beyond Color Stage 5, the dehydroascorbic acid increases at the expense of the reduced and there is some decrease in the total.

Cooking of partially ripe fruit, in the skin, either by baking or boiling, destroys about 10 per cent of the vitamin C; fully ripened fruit loses 50 to 75 per cent.

Bromine was a suitable oxidant to convert ascorbic into dehydroascorbic acid.

The carotene content of plantain is about 0.0003 per cent, irrespective of the stage of ripeness. If this were all β-carotene, it would represent about 500 I. U. per 100 gm. of fresh pulp.

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ULTRAVIOLET IRRADIATION OF BEEF AND PORK FOR LOW TEMPERATURE STORAGE[1]

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Information on the use of germicidal lamps to control microbial growth on the surfaces of beef carcasses being rapidly aged in controlled "high temperature-high humidity" chambers was first made available by research work completed at the Mellon Institute. The process of rapid tenderization proceeds as a result of high temperature which speeds up the natural enzymatic processes occurring in the tissues. The high humidity eliminates shrinkage as a problem. The maintenance of high temperatures and high humidities, however, stimulates rapid microbial growth which, if not controlled, results in heavy trimming losses.

Frozen food locker plants have, in many cases, installed germicidal lamps to assist in controlling infection as well as the resulting odor which develops in chill- and age-rooms. For various practical reasons it is not feasible to use high temperature aging in the locker plant. It is, therefore, only because the ultraviolet irradiations will prolong the life of the chilled product that it can be considered to facilitate the aging process.

It seems desirable to mention that prolonged aging is considered by many researchers, as well as operators, to contribute little to increased tenderness of the frozen product and possibly to have a deleterious effect in shortening the rancid-free lifetime of the frozen product. It is an established fact that certain ultraviolet irradiations shorten the induction period to rancidity development. This may be due to emissions below 2000Å and resultant ozone generation. The problem is, therefore, possibly concerned not so much with the likelihood of such an occurrence but rather with whether the development is of such magnitude as to be of practical importance. The experimental work reported in this paper is concerned solely with this characteristic and not with such problems as the efficiency of irradiation as a germicidal agent.

Rentschler, Nagy, and Mouromseff (1941) report results from rather extensive investigations on the bactericidal action of ultraviolet irradiation which demonstrate the value of ultraviolet irradiation as a bactericidal agent.

Mallman and Churchill (1946) found that ultraviolet rays produced a reduction of bacteria on surfaces directly irradiated, followed by a gradual increase after prolonged irradiation. Surfaces shielded from direct

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irradiation in closed rooms showed some reduction in surface populations. Ozone atmospheres gave results comparable to those obtained with shielded ultraviolet rays.

Hollaender (1942) reports that bacteria and fungi show a maximum sensitivity to ultraviolet irradiation at 2650Å wavelength.

Ewell (1939, 1941, 1942, 1946) reports that a concentration of ozone of a few 10ths of one p.p.m. found in the air irradiated by lamps with major output at 2537Å inhibits mold and bacterial growth on the surfaces. Where ozone alone is added to the air the concentration must be above one p.p.m. to be effective. Fats often develop rancidity in atmospheres containing a concentration of one p.p.m. In order to protect air pockets and surfaces not reached by direct irradiation of the ultraviolet lamps, it is necessary to rely on the accompanying production of ozone. This means the glass envelope must transmit a small amount of irradiation at 1850Å in order to synthesize a portion of the oxygen in the air into ozone. The amount of ozone required per hour will depend on size of the room, surfaces of stored meat, humidity, room temperature, and velocity of air movement. This amount of ozone can be obtained by a proper proportion of "high 1850Å transmission" lamps to those having very little output below 2000Å.

Holm, Greenbank, and Deysher (1927), in discussing the catalytic action and other factors, state the induction period to rancidity is shortened by ultraviolet light at 2537Å.

Fredholm (1946) reports that in work with three types of high pressure lamps irradiation decreased the rate of bacteriological decomposition by 30 per cent on meat products, but that the rancidity of the fat was increased by direct irradiation. Storing fat in an atmosphere of irradiated air gave no increase in rancidity. Small quantities of ozone formed in the experiments had no influence on the velocity of the reactions.

McIntosh, Sotola, Boggs, Roberts, Prouty, and Ensminger (1942) have shown that beef short loins aged seven to 14 days at 1.1 to 10°C.(34 to 50°F.) and at 90 per cent relative humidity with ultraviolet rays of 2537Å showed a reduced surface microflora. The irradiated steaks showed greater rancidity than the non-irradiated. However, Sotola, McIntosh, Prouty, Dobie, Ensminger, and McGregor (1943) report loin steaks irradiated 147 hours at 2537Å at 1.1 and 10°C. showed no rancidity that could be detected by either peroxide or taste tests.

Brady (1942) compared prime ribs of beef irradiated two and four days at 10°C.(50°F.) where the major output was at 2537Å, with ribs aged 10 and 20 days at 1.1°C.(34°F.) without irradiation. The roasts were frozen and stored at —17.8°C.(0°F.). None of the roasts was found to contain rancid flavors regardless of length of aging, whether irradiated or not, even after 10 months of storage at —17.8°C.

METHODS

Studies were carried out on carcasses of hogs and beef cattle grown and fattened on the North Carolina Agricultural Experiment Station farm. A special effort was made to insure uniformity by selecting only animals of similar breeding and feeding histories. All of the irradiated,

as well as check carcasses, were aged at approximately 2.2°C.(36°F.) and at 90 per cent relative humidity. After cutting, samples were wrapped in waxed freezer paper, quick frozen, and stored at —17.8°C. Back fat samples consisted of strips three inches wide and one-half inch thick, taken between the fourth thoracic and third lumbar vertebrae.

Germicidal lamps were installed according to the manufacturer's recommendations. Tests by an independent electrical testing laboratory showed the type germicidal lamps used to have an average erythemal energy output of 71.6. Approximately 90 per cent of the energy intensity was at the 2537Å spectral line with no energy output below 2000Å. Since this study was concerned with ultraviolet irradiation it was considered inadvisable to complicate procedures by using irradiation below 2000Å and thus generating appreciable amounts of ozone. The erythemally weighted ultraviolet energy output of the lamps was determined at a point 12 inches from the light center on the lamp axis, and computed from these measurements on the basis of data published by Coblentz *et al.* (1932).

Methods for determining peroxide values were the same as described by Smith, Brady, and Comstock (1945), except the fat samples were heated in an oven at 75°C.(167°F.) for two instead of three hours. Peroxide values are expressed as milli-equivalents of peroxide/kg. of fat. Free fatty acids were determined by the official A.O.A.C. method (1935). Free fatty acid values are expressed as per cent oleic acid. The iodine absorption numbers were determined by the Hanus method, A.O.A.C. (1940). The terms "aging" and "holding" are used interchangeably.

EXPERIMENTAL PROCEDURE

Experiment 1. *Effect of irradiating pork carcasses 24 and 48 hours on development of peroxides and free fatty acids.*

Left sides of five carcasses were irradiated 24 hours and the right sides 48 hours. Five other carcasses were used as checks. In addition to the initial iodine values, peroxide and free fatty acid determinations were made of the leaf and back fat samples at three-, six-, and 12-month intervals. The 12-month values are listed (Table 1).

TABLE 1

Effect of Irradiation on Development of Peroxides and Free Fatty Acids in Pork Fat After 12 Months' Storage at —17.8°C.

Treatment	Iodine number	Peroxide number		Free fatty acid	
	Back fat	Back fat	Leaf fat	Back fat	Leaf fat
				pct.	*pct.*
Irradiated 24 hours	51.85	1.84	2.20	0.92	0.53
Irradiated 48 hours	52.46	0.88	3.80	0.46	0.44
Check 24 hours	54.25	3.00	4.12	1.26	0.45
Check 48 hours	54.70	0.76	5.64	0.65	0.67

The extremely low peroxide and free fatty acid values obtained will be noted (Table 1). There is no discernible direct relation between peroxide values and irradiation. Length of aging period had no direct effect on the peroxide and free fatty acid formation. On the average, the peroxide

and free fatty acid values increased steadily and significantly [4] over the storage period, notwithstanding the small magnitude of the 12-month storage values. Leaf and back fat samples gave somewhat different trends over the 12-month storage period.

Experiment 2. *Effect of prolonged chill temperature holding on the development of peroxides and free fatty acids in frozen pork fat.*

Strips of back fat three-fourths-inch wide and one-half-inch in thickness were taken from two pork carcasses after 48-, 96-, 144-, and 192-hour holding intervals. The iodine numbers for the back fat were 68.1 and 67.4.

TABLE 2

Effect of Length of Holding Period on Development of Peroxides and Free Fatty Acids in Non-Irradiated Pork Back Fat After Storage at —17.8°C.

Days stored	Peroxide number (Hours held)				Free fatty acid (Hours held)			
	48	96	144	192	48	96	144	192
					pct.	*pct.*	*pct.*	*pct.*
89	3.0	3.0	3.2	4.0	.32	.23	.42	.26
117	1.8	0.0	1.2	2.8	.20	.26	.33	.40
145	2.8	3.6	2.4	4.2	.33	.36	.42	.50
176	4.8	4.0	3.0	4.4	.23	.20	.25	.51
203	4.4	2.0	3.8	6.4	.60	.46	.55	.42
232	12.2	10.2	15.8	19.2	.51	.42	.53	.60
286	8.4	7.8	8.4	12.0	.40	.50	.58	.60
323	12.8	9.8	16.0	19.6	.44	.66	.92	.68

TABLE 3

Effect of Irradiation on Development of Peroxides and Free Fatty Acids in Pork Fat After 12 Months' Storage at —17.8°C.

	Iodine number	Peroxide number irradiated		Free fatty acid irradiated	
		48 hours	144 hours	48 hours	144 hours
				pct.	*pct.*
Back fat	54.71	2.10	5.44	.69	.84
Leaf fat	47.54	5.26	12.56	.65	.78

There is some indication (see Table 2) that the longer holding periods increased the rate of development of peroxides and free fatty acids. No significant differences could be found, however, on length of period. It is quite possible that had more carcasses been sampled a difference would have been noted. Highly significant effects were found relating length of storage period to both peroxide and free fatty acid values.

Experiment 3. *Effect of prolonged irradiation on development of peroxides and free fatty acids in frozen pork fat.*

Right sides of six pork carcasses were exposed to irradiation for 48 hours and the left sides for 144 hours. Samples were analyzed at the end of three-, six-, and 12-month periods. Peroxide and free fatty acid values

[4] The term "significant" is used in the sense of being statistically significant.

obtained after 12 months' storage are given (Table 3) together with the iodine values.

On an average, peroxide values were appreciably higher for the leaf fat than for the back fat. (This is possibly associated with the greater surface area exposed.) Irradiation for the longer period gave higher peroxide and free fatty acid values. These differences were highly significant although not of great magnitude in the case of the free fatty acid content.

Experiment 4. *Effect of irradiation on aging of beef.*

Right sides from 12 good-to-choice cattle carcasses were aged seven days, and left sides 14 days. Six left and right sides were irradiated and the remaining sides were not. At the completion of the aging period roasts consisting of the 11th and 12th ribs were removed, wrapped in waxed freezer paper, over-wrapped with kraft paper, and frozen. Roasts from half the carcasses on each treatment were tested after six months' storage; the other half after 12 months.

TABLE 4

Effect of Length of Holding Period and Irradiation on Development of Peroxides and Free Fatty Acids in Beef Fat Stored at —17.8°C.

Length of storage	Peroxide number		Free fatty acid	
	6 months	12 months	6 months	12 months
			pct.	*pct.*
Irradiated 7 days	0.32	1.26	0.92	1.75
Irradiated 14 days	3.10	5.26	1.39	1.57
Non-irradiated 7 days	0.00	5.50	0.67	1.12
Non-irradiated 14 days	0.44	5.54	1.38	1.10

While the results (Table 4) seem to indicate irradiation increases the peroxide values, the differences were markedly short of being significant. Longer aging resulted in a marked and highly significant increase in peroxide values.

Of interest was the fact that 14 days of irradiated aging produced the highest peroxide values, while seven days of irradiated aging produced the lowest values after 12 months' storage. On the average, the increase in both free fatty acid and peroxide values between the six- and 12-month storage period, while not large, was highly significant.

In addition to the peroxide and free fatty acid determinations a palatability committee scored the roasts which had been cooked to an internal temperature of 56 to 58°C.(132.8 to 136°F.). Factors considered were intensity of aroma, lean and fat flavor, texture, tenderness, juiciness and desirability of lean flavor, and cooking losses. Results indicated the following significant differences:

(1) Greater juiciness after six than after 12 months' storage.
(2) Greater desirability of aroma after six than after 12 months' storage.
(3) Greater evaporation loss on beef aged 14 days than that aged seven days. This difference was small after six months, but was greater after 12 months and especially marked with irradiated beef.

(4) Higher cooking drip loss in beef stored for 12 months than for beef stored six months.

(5) Flavor, aroma, and desirability characteristics of the roasts were not discernibly different for the several treatments and showed little, if any, relationship to peroxide and free fatty acid values.

DISCUSSION AND CONCLUSION

Results of this study provide answers to some of the questions which have been raised concerning ultraviolet irradiation of meat which is to be frozen. While these results are limited to conditions stated previously, nevertheless their application might be considered to hold on a somewhat more general basis. Apparently irradiation at 2537Å has no deleterious effects providing the carcasses are not held for greater lengths of time than those commonly recommended. Irradiation of pork carcasses for 24- to 48-hour periods likewise cannot be considered to have any deleterious effect. This is especially true since in the present study these fats were packaged in materials that are not good oxygen barriers and were stored for a full 12 months' period. In Experiment 2 it is suggested that extremely prolonged aging (up to 192 hours) may have shortened the storage lifetime of the frozen pork. In Experiments 1 and 2 length of frozen storage had profoundly more effect on the peroxide number than length of the pre-freezing holding period. Holding pork 192 hours prior to freezing resulted in peroxide values of appreciable size, but these were of insufficient size to indicate organoleptic rancidity. Experiment 3 definitely indicates prolonged aging results in higher peroxide values after storage for one year. These differences, while highly significant, were not of sufficient size to indicate a deleterious effect on the palatability.

Effect of irradiation on beef was even less pronounced than in the case of pork. Irradiation tended to increase peroxide values, but the values for all treatments were low. Longer aging resulted in higher peroxide values, but these were still far too low to indicate an effect on the palatability of the meat.

In discussing results for both beef and pork fat analyses it should be emphasized that the peroxide and free fatty acids were never of sufficient size to indicate discernible rancidity by taste test. There were computed for the irradiated samples estimates of limits below which 95 per cent of the samples would fall in repetitions of these experiments. For the peroxide number on the back fat in Experiments 1 and 3 at 12 months' storage the limits were 2.3 and 5.1, respectively. Corresponding values for leaf fat were 6.2 and 11.7, respectively. For the beef fat at six and 12 months the limits of peroxide numbers were 1.75 and 4.86, respectively.

On the basis of these experiments it may be concluded that length of frozen storage period had the most pronounced effect on rancidity development. The effect of irradiation was of little importance and length of the holding or aging period of intermediate influence. Peroxide values found in these studies after 12 months of frozen storage were not of sufficient magnitude to indicate discernible organoleptic differences. Substantiation of this point was found with the palatability tests. It cannot be assumed, however, that the use of germicidal irradiation of wavelengths shorter than

those used in these experiments would be without effect in stimulating production of peroxides.

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SOME EFFECTS ON SWELLING POWER OF WHEAT STARCH OF (1) SOXHLET EXTRACTION WITH METHANOL AND (2) COMPOSITION OF THE COOKING LIQUID

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This work is a continuation of the studies on the swelling power of cereal starches described by Harris and Jesperson (1946) and Harris and Banasik (1948), with emphasis on the effect of methanol extraction on swelling power and the influence of electrolyte concentration in the cooking liquid.

EXPERIMENTAL PROCEDURE

Materials: The starch sample used was prepared from hard, red, spring wheat flour by washing from a dough and separating the water by centrifuging. The starch was washed several times with distilled water and dried to a moisture content of about 13 per cent.

Methods: The fat was removed by refluxing in 100 per cent methanol for 24 hours using a Soxhlet fat extraction apparatus. Schoch (1942) has indicated the efficiency of this reagent in the removal of lipid material from starch, although he employed a concentration of 85 per cent. After drying the fat was determined by the acid hydrolysis method. This method consists, in brief, of heating 100 grams of starch in 250 ml. of two N hydrochloric acid in a 600-ml. beaker in a steam bath for from two to three hours with stirring. After cooling and standing in a refrigerator overnight the mixture is gravity-filtered, using a fluted filter paper. After carefully cleaning the beaker and adding any traces of fat found to the residue, the latter is dried in a warm place [below 60°C.(140°F.)] and subsequently in a vacuum oven at 50°C.(122°F.). The paper and contents are then extracted in a Soxhlet with petroleum ether for eight hours. The ether is then evaporated, the flask dried in vacuum, cooled and weighed. The weight of the residue yields per cent fat ("as is" basis). Moisture content was determined by heating three grams of starch in an electric drying oven for one hour at 130°C.(234°F.), and the protein content by the Kjeldahl-Gunning method, employing three grams of starch. Phosphorus content was obtained by the procedure described by Koenig and Johnson (1942), which depends upon the transmission of light through a liquid containing a phosphovanadiomolybdate compound.

For removal of residual methanol from the starch which had been extracted, the sample was allowed to stand under room conditions until the odor of methanol had disappeared. The starch was then heated in a vacuum oven at approximately 55°C.(131°F.) for 24 hours under a vacuum of about two inches of mercury. Finally, the sample was rehydrated by exposure to an atmosphere of 78 per cent relative humidity

at 30°C.(86°F.) for 96 hours. The final moisture content was 15.5 per cent. The starch regained some of the general appearance of the original, non-extracted sample.

The starch granules were swollen by heating in water for five minutes in 50-ml. centrifuge tubes in a Dewar flask at the selected temperatures. Heating was accomplished by immersion heaters, one of which was connected to a suitable rheostat, for controlling power input and thus the temperature, Harris and Banasik (1948). The procedure was that also described by these workers; it is of course empirical and measures water in the granules, water adsorbed on them, and water mechanically held between them. "Swelling power" and "swell" are employed synonymously and imply the quantity of water in grams taken up by one gram of dry starch.

RESULTS

Effect of Methanol Extraction: The moisture, protein, phosphorus, and fat contents of the original and extracted samples are shown in the following table:

	Moisture	Protein	Phosphorus	Fat
	pct.	*pct.*	*pct.*	*pct.*
Original	13.20	0.30	0.07	0.44
Methanol-extracted	9.80	0.22	0.05	0.11

It is apparent that the fat has been reduced to one-fourth its previous value by the treatment with methanol. Phosphorus and protein contents were also reduced slightly.

The two starches were heated in redistilled water and in dilute solutions of aluminum sulfate to 60°C.(140°F.), 70°C.(158°F.), 80°C.(176°F.), 90°C.(194°F.), and 95°C.(203°F.), (Fig. 1). These data show that methanol extraction increased the swelling power in both water and 0.001 N $Al_2(SO_4)_3$ solution, particularly at the higher temperature range. The curves show the familiar relationships between temperature and swelling power.

The curves from the starch extracted with methanol appear smoother than those from the original starch, and lack the pronounced inflection in the neighborhood of 80°C.(176°F.). This is probably caused by facilitating the entry of water molecules into the extracted starch at lower temperatures. Even at 60°C.(140°F.) methanol induced greater swell.

There was also a distinct change in the physical appearance of the starch following extraction. Hites, Sandstedt, and Gates (in press) have shown that methanol extraction has a marked effect on the gelatinizing properties of starch, but reported no alteration of the photomicrographic appearance of the granules. The effect of swelling power could be attributed to either partial removal of the fat, which would facilitate the entry of water molecules, or to some variation induced in the chemical relations of the starch structure.

Treatment of the extracted starch for removal of the methanol had little effect on the swelling power, but the results were consistently higher than for the original starch. This suggests that the principal effect of

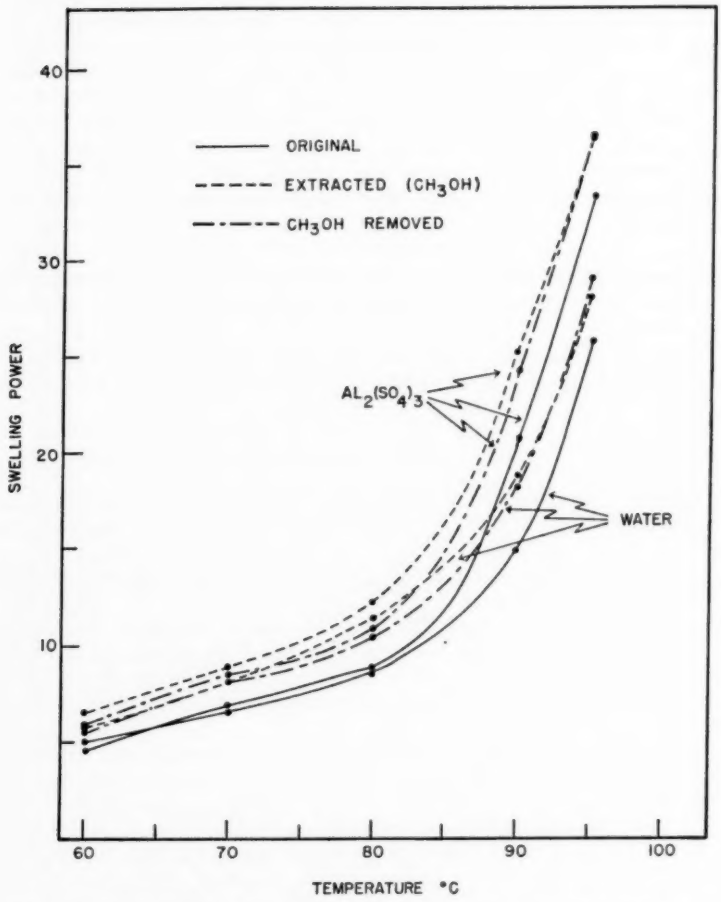

FIG. 1. Effect of extraction with methanol on the swelling power of wheat starch when cooked in redistilled water and in a solution of 0.001 N $Al_2(SO_4)_3$. Effect of removal of residual methanol from the extracted starch is also shown. Swelling power represents the grams of water associated with one gram of dry starch.

the methanol was exerted through the removal of lipid material, with the specific influence of the methanol itself playing a minor role. More data are required to definitely settle this point. Results with $Al_2(SO_4)_3$ solution were all above those secured with redistilled water at 90 and 95°C. (194 and 203°F.).

That methanol extraction increases the swelling power in electrolyte solutions in much the same fashion as in unextracted starch is shown (Table 1). The difference in swelling power between starches cooked in H_2O and $Al_2(SO_4)_3$ solution is approximately the same as between the mean values for all cooking liquids of the original and extracted starch. Also, the effect of methanol in increasing swell appears to decrease as the swelling power induced by the presence of an electrolyte in the cooking liquid increases. Thus the increase in $Al_2(SO_4)_3$ solution due to methanol is 4.5, while in $K_4Fe(CN)_6$ it is 6.1. These differences may be fortuitous, but they create the impression that the effect of methanol is related to the composition of the cooking liquid.

TABLE 1

Effect of Methanol Extraction on Swelling Power of Wheat Starch at 90°C.

Cooking liquid	Original	Methanol-extracted
$Al_2(SO_4)_3$	20.7	25.2
Water (redistilled)	14.9	18.9
K_2SO_4	14.4	19.0
KCl	13.6	19.2
$K_4Fe(CN)_6$	12.6	18.7
Mean	15.2	20.2

Effect of Electrolyte Concentration: Information on the effect of concentration on swelling power of unextracted starch for seven electrolytes is provided (Table 2). In addition to the mean swelling power, values for

TABLE 2

Effect of Electrolyte Solutions at Various Concentrations on Swelling Power at 90°C.[1]

Electrolyte used	Normality of solution					Mean
	0.001	0.003	0.005	0.007	0.010	
$Al_2(SO_4)_3$	19.3	20.3	20.8	21.3	21.5	20.6
K_2SO_4	15.4	14.4	14.2	14.2	14.0	14.4
$MgSO_4$	13.9	14.2	14.4	13.0	13.6	13.8
KCl	13.2	14.4	13.8	13.4	13.4	13.6
$Na_3C_6H_5O_7$	13.9	13.0	13.4	14.0	12.1	13.3
$K_4Fe(CN)_6$	15.1	13.2	13.2	12.7	11.5	13.1
$K_3Fe(CN)_6$	13.9	12.5	12.8	12.3	11.6	12.6
Mean of treatments with original starch samples	15.0	14.6	14.7	14.4	14.0	.....
Mean of treatments with original and extracted starch samples	16.0	16.0	16.1	15.6	15.5	.....

[1] Data arranged in order of decreasing mean swell.

both original and extracted starch are shown at the foot of the table. Since there were only three series of concentration tests made with methanol-extracted starch, the original data from the unextracted samples only are included. Apparently there is little significant effect, although there is some indication in the mean or average values that there is an inverse relationship between concentration and swelling power. Further data are needed to definitely settle this point. The methanol-extracted sample yielded no evidence of any effect by concentration, but since only three electrolytes were included in this part of the experiment the means are not shown. Aluminum sulfate caused the greatest swell and was much more effective than any other salt used. This effect appeared to be due to the Al-ion, since the SO_4-ion exerted little influence on the results, and K_2SO_4 and $MgSO_4$ induced rather similar swell, which was much lower than the swell caused by $Al_2(SO_4)_3$. Harris and Banasik (1948) have reported similar results. Magnesium, potassium, and sodium ions had approximately equal effects. The SO_4-ion seemed to be rather more effective than either the ferro- or ferri-cyanide ion.

SUMMARY

Methanol extraction in a Soxhlet extractor decreased the fat content of wheat starch approximately 75 per cent and increased the swelling power of the starch when cooked at temperatures from 65 to 95°C.(149 to 203°F.). This increase occurred in water and in electrolyte solution, and there was some evidence the increase was inversely related to the efficiency of the electrolyte in promoting swell. Removal of the methanol very slightly decreased the swelling power.

Electrolyte concentration in the cooking solution had a rather minor influence on swelling power, although there was an indication that increasing the concentration to 0.01 N tended to slightly depress swelling power at 90°C.(194°F.).

A brief discussion of the possible mechanism by which methanol extraction affects swelling power is included.

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RAPID DETERMINATION OF COLLAGEN IN BEEF BY WARING BLENDOR AND CENTRIFUGE TECHNIQUE[1,2]

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Bell, Morgan, and Dorman (1941) have demonstrated that the Mitchell, Hamilton, and Haines (1928) method for determination of collagen in meat may fail to account for as much as one-half of the collagen.

While the method of Bell *et al.* (hereafter called the Bell method) demonstrated a greatly improved reliability, it unfortunately added a greater burden to the already tedious and time-consuming method of Mitchell *et al.* (hereafter called the Mitchell method).

Cover, Mackey, Murphey, Miller, Bass, Bell, and Hamalainen (1944) have compared collagen analyses obtained by using modified methods of Bogue (1923) and of Spencer, Morgulus, and Wilder (1937). They have found distinctly higher results by the former method, due to its inclusion of other soluble nitrogen compounds. The Spencer method (a gelatin-tannate procedure), while apparently reliable, is likewise burdened with an excessively time-consuming procedure.

The senior author and co-workers (1944) have observed that non-turbid aqueous extracts of raw meat could be obtained by centrifugation of aqueous dispersions of beef muscle tissue adjusted to the isoelectric point (pH 5.07). Therefore, it seemed it should be possible to substitute a rapid centrifugation operation in place of the sieve and linen filter procedure of the Bell method.

Furthermore, it seemed that the 90-minute ball-milling step used in both the Bell and Mitchell methods might be substituted for by use of the Waring Blendor. Preliminary experiments had shown that the Waring Blendor dispersed beef muscle tissue so finely that it nearly all passed quite easily through a 100-mesh sieve. Such a procedure for dispersion would, therefore, be impractical for the sieve and linen filter separations employed by the Bell method. However, such fine dispersion should offer no difficulty in centrifugation, and, in fact, should aid in more effective extraction than that attained by ball-milling.

Since Hall *et al.* (1944) and many other workers have observed a relation between tenderness of meat and collagen content, it is evident that a method for collagen determination employing simple, expeditious technique of the kind proposed would be highly useful in meat research.

[1] Contribution No. 379, Department of Chemistry, Kansas Agricultural Experiment Station.

[2] From thesis submitted by Lois Meisner Hartley in partial fulfillment of the requirements for the degree of Master of Science in Home Economics, Kansas State College. This work was supported by a scholarship made available from funds granted by the National Live Stock and Meat Board, Chicago, Illinois.

MITCHELL, HAMILTON, AND HAINES METHOD

The Mitchell water extraction method for collagen used in this investigation depends upon the separation of water-soluble proteins by means of a 100-mesh sieve. The 100-gram samples were placed in ball mills with 300 ml. of water and ground with two pounds of balls for 90 minutes, then washed on a 100-mesh sieve. Seven washings were used, as recommended; each time the residue from the sieve was taken up in a beaker with 150 to 300 ml. of water, 45 to 50°C.(113 to 122°F.), and stirred with a glass rod until completely dispersed. All the filtrates were rejected. Following the seventh washing the residue was dispersed in a 800-ml. beaker with 400 ml. of water and autoclaved two hours at 15 pounds pressure. The supernatant liquid was decanted through a linen filter and the residue washed five times by decantation with 100 ml. of boiling water, and finally transferred to the filter; the residual liquid was removed by suction. The combined filtrate and washings were made to a liter volume with distilled water, and 100-ml. aliquots were taken for nitrogen determination by the Kjeldahl method.

BELL, MORGAN, AND DORMAN METHOD

In attempting to apply the Mitchell method of collagen determination to cooked meat, Bell *et al.* (1941) noticed a large amount of granular material which passed through the 100-mesh sieve each time the tissue from the ball mill was washed. When this granular material was recovered from the filtrate, washed free of the water-soluble protein, and recombined with the residue on the sieve, a considerable increase in collagen nitrogen was noted. These observations were confirmed in this laboratory.

Following the autoclaving of the combined residues from the sieve and the Buchner funnel, the liquid was tested for tryptophane. Negative results were obtained showing no proteins other than gelatin present in the filtrate. The Bell method gave more consistent results for collagen than did the Mitchell method, in which the granular residue was discarded.

The filtrate from the autoclaved residue was concentrated to 500 ml. It often appeared cloudy and was cleared by adding 10 ml. of 0.1 *N* H_2SO_4, refluxing on a steam bath for two or more hours, and filtering before the aliquot was taken.

The cooked meat was found to contain less collagen than the samples of raw meat. The collagen apparently was hydrolyzed during cooking, and the hydrolysis appeared to be greater during the longer cooking periods. It was concluded that the method as described could be used as a reliable measure of change in tenderness caused by cooking.

However, the method was still laborious, unwieldy, and time-consuming. A more rapid method is necessary in following on an extensive scale the change in structure and tenderness of meat during cooking, storage, freezing, or aging.

CENTRIFUGE METHOD

Observations made by Hall *et al.* (1944) concerning water imbibition and isoelectric point showed that when meat in a suspension of water is brought to its isoelectric point, the non-soluble proteins may be separated from the liquid by centrifuging. This fact suggested a new method of

separation for collagen from the other meat proteins without involving the use of the sieve.

Duplicate 10-gram samples of ground beef tissue were homogenized for five minutes in a Waring Blendor with 40 ml. of ice water and the previously determined amount of normal sulfuric acid to adjust the meat to its isoelectric point (pH 5).

Adjustment of pH: In order to determine the amount of normal sulfuric acid necessary to adjust the meat suspension to pH 5, a separate assay for that purpose was made. The pH of a blended sample was determined with a glass electrode potentiometer. A tentative amount (0.2 to 0.4 ml.) of the acid was blended with the meat and water. The pH was again determined, and by proportion the theoretical amount of acid was calculated, added to a fresh portion of meat, and mixed. The pH was again determined and further adjustments made if necessary.

The homogenate was centrifuged and washed by centrifugation with warm water.

A biuret test, Hawk, Oser, and Summerson (1947), was made upon the final washing to insure the removal from the residue of all water-soluble proteins. The fifth washing was negative for the raw sample. A sixth washing was found to be necessary for the cooked sample.

TABLE 1

Change in pH of Successive Series of Washings of Homogenized Beef Rump Muscle Tissue

pH of tissue	5.46
pH after homogenizing with 0.20 ml. N/1 H_2SO_4 per 10 gm.	5.00
pH of first decantate	4.99
second	4.50
third	4.48
fourth	4.41
fifth	4.39
sixth	4.39

The pH of a typical series of washings is shown (Table 1). There was a surprising increase in acidity, especially in the second decantate. However, the washings remained free of turbidity. Otherwise, further pH adjustment might have been necessary to maintain an isoelectric value.

The residue was dispersed with 80 ml. of water in a 125-ml. Erlenmeyer flask and autoclaved two hours at 15 pounds pressure. The autoclaved material was centrifuged and washed, and the supernatants poured into a Kjeldahl flask. The fifth washing gave a negative biuret test. The residue was discarded and the entire decantate was used for collagen nitrogen determination by the Kjeldahl method. Collagen nitrogen was expressed as per cent of total nitrogen.

Tryptophane tests were made on several autoclaved samples to assure complete removal of non-collagen proteins by the procedure prescribed by Bell *et al.* (1941). No measurable amount of tryptophane was found to be present.

EXPERIMENTAL RESULTS

A sample of round was selected to be used in the determination of collagen in raw beef by the three methods previously described. Com-

parison of the data indicated that the Bell and centrifuge method both gave results higher than those obtained by the Mitchell method (Sample 1, Table 2). However, the results by the Mitchell method did not deviate

TABLE 2

Adjustment of pH for Centrifuge Method for Collagen Determination. Comparison of Collagen Nitrogen Determined by the Centrifuge, Bell, and Mitchell Methods

Sample No.	Natural pH	Normal [1] H_2SO_4 ml.	Collagen nitrogen in per cent of total nitrogen		
			Centrifuge	Bell	Mitchell
Raw sample					
			pct.	*pct.*	*pct.*
1	5.46	0.20	11.70	11.40	9.80
2	5.58	0.32	13.40	13.40	.....
3	6.35	0.72	9.10	11.60[2]	.....
4	5.68	0.28	13.90	10.60	.....
Cooked sample					
4	5.92	0.56	12.20	9.23	.....
5	5.78	0.36	11.20	9.65	.....
6	5.74	0.28	10.80	8.10	.....

[1] Volume required to adjust 10-gram sample to isoelectric point (pH 5.0). [2] After adjustment to pH_5.

so widely from the other methods as was reported by Bell *et al.* (1941), who found differences between the two methods amounting to 50 per cent. Closer agreement between the results obtained by the two methods in this laboratory indicated that the Mitchell method is subject to considerable deviation in results due to the variation of individual manual technique in different laboratories.

TABLE 3

Reproducibility of Results Obtained by the Centrifuge Method for Collagen in Beef Round

Determination No.	Collagen nitrogen in per cent of total nitrogen
	pct.
1	11.1
2	11.9
3	11.9
4	11.1
5	11.9
6	11.7
7	11.7
Average	11.7
Average deviation from mean	0.2

In order to test the reproducibility of the centrifuge method, a series of samples of the same round was analyzed. Collagen content for the series is shown (Table 3). It was concluded that reproducible results could be obtained by the centrifuge method to determine the collagen nitrogen of raw beef.

Previous experience in this laboratory has indicated that the Mitchell method did not give satisfactory duplications. For example, in a series of 11 samples differences in duplication ranged from zero to 1.5 per cent collagen nitrogen, with an average of 0.6. Differences in duplication by the Bell method on three raw samples averaged 0.4 per cent, and on three cooked samples, 0.2 per cent. For the centrifuge method the differences averaged 0.3 per cent on four raw samples and 0.8 per cent on three cooked samples.

Results by the centrifuge method tended to run somewhat higher than those obtained by the Bell method. This difference may be explained by the fact that more effective separation of water-soluble protein was obtained at the isoelectric point by the centrifuge method. Although the Bell method recovered much of the residue passed through the sieve by the Mitchell method, it was not all retained by the linen filter.

Dark-Cutting Beef: Because of the physical differences between dark-cutting and normal beef, a dark sample was included in an experiment set up to determine the collagen content by the Bell and by the centrifuge method. Hall *et al.* (1944) described dark-cutting beef and its characteristic physical properties, as well as some chemical properties, especially its high pH. The color of the lean might range from brownish-red to purplish-black, with no abnormal appearance of the fat tissue; but the texture of the beef was always sticky and gummy. Difficulty was encountered when an attempt was made to separate the water-soluble proteins from the collagenous tissue. The normal procedure for the Bell method was followed in which 100-gram portions of meat were ball-milled with 300 ml. of water. When the samples were washed onto the sieve, the fibers were matted together with a gelatin-like material which could not be separated. Washings were attempted, but when a sixth washing was completed a reddish tinge persisted in the tissue, and fibers were still bound together by the gelatinous mass. An attempt was made to filter the colloidal suspension which passed through the sieve, as directed by the Bell method. The suspension was much too colloidal and passed through the linen filter, making it impossible to separate the granular residue. It was evident the method was entirely unsatisfactory for the collagen determination of dark-cutting beef.

Therefore, the Bell method was modified to the extent of adjusting the tissue to pH 5 before the ball-milling process. Filtering and washing processes then yielded clear filtrates and the analysis was completed without difficulty. Results are indicated (under Sample 3, Table 2).

When the dark-cutting beef was extracted by the centrifuge method no difficulty in separation occurred. The residue packed firmly in the bottom of the centrifuge tube and the filtrate was clear and drained easily each time a washing was made. The residue appeared white, with no trace of a reddish tinge. The autoclaving and washings were conducted as described.

Comparison of the data obtained revealed that in this case alone the modified ball-milling process gave a somewhat higher collagen nitrogen, 11.6 per cent, than the centrifuge method, which gave 9.2 per cent collagen

for the same sample. The significance of such an anomalous sample, however, cannot be estimated.

Cooked Beef: The centrifuge procedure was applied to the determination of collagen in cooked beef. Physical and chemical changes in the proteins brought about by the cooking process evidently altered the isoelectric point and the pH.

The pH was varied in a series of samples of braised beef by adding increments of 0.05 ml. of normal sulfuric acid. The point of minimum water imbibition was taken as the isoelectric point, as described by Hall *et al.* (1944). The pH points fell within one-tenth unit apart and the isoelectric points varied within approximately 0.2 unit. When the pH range was sufficiently extended, two minima appeared in the water imbibition curve, one falling closely to the value for raw beef muscle (5.07) found by Hall *et al.* (1944). The second point in all cases was higher, ranging from 5.2 to 5.4. The pH of three cooked samples was 5.67, 5.74, and 5.92, considerably higher than the normal pH of raw beef. The sample having the highest pH had the lowest isoelectric point; therefore, it does not seem feasible to predict the amount of acid required to reach the isoelectric point from the pH of the cooked beef.

The isoelectric point of the cooked beef ranged from pH 5.18 to 5.40. Therefore, it seemed advisable to adjust the pH of cooked beef to a point somewhat higher than the isoelectric point of raw beef. A pH of 5.2 may be selected as a trial value which should not be far from the average isoelectric point of the altered and unaltered proteins. After centrifuging, if the supernatant is turbid, further adjustment of pH may be necessary.

In all instances in which the Bell method was used for cooked beef analysis considerable difficulty was encountered in filtration due to the colloidal nature of the ball-milled material. It was necessary to clear the autoclaved filtrate with H_2SO_4 for a similar reason. It was found the colloidal material could be coagulated by adding H_2SO_4 before evaporation to volume, thus avoiding refluxing.

When the centrifuge method of determining collagen nitrogen was applied to cooked beef the procedure used for raw beef was followed, with the exception of adjusting the pH to 5.2 and the use of an additional washing of the homogenized material.

The collagen nitrogen yield of the round by the Bell method indicated a 10.95 per cent decrease in collagen content during the braising process. When analyzed by the centrifuge method the decrease was 11.6 per cent.

Bell *et al.* (1941) reported considerably higher conversion of collagen to gelatin by cooking than shown by the data indicated here. The variation is probably due to the different methods of cooking. The method of simmering, or boiling in excess water, used by Bell may convert more of the collagen than the method of braising, in which a small amount of water was used.

SUMMARY OF THE CENTRIFUGE METHOD

The method for the determination of collagen involving the use of the centrifuge as finally developed is essentially as follows:

Preparation of Sample: After the external fat and visible cartilage have been removed, put the meat twice through a hand-grinder, first with

a coarse blade and then with a fine blade, and mix thoroughly. Weigh duplicate 10-gram portions of ground sample into small-size, chilled Waring Blendor jars, add 40 ml. of ice water with the normal H_2SO_4 necessary to bring the suspension to the desired isoelectric point (pH 5.0 for raw; pH 5.2 for cooked), and homogenize for five minutes.

Water Extraction: Rinse the homogenate into a 250-ml. Erlenmeyer beaker and allow it to stand for 10 minutes. Decant the liquid into a 40-ml. centrifuge tube, centrifuge five minutes at 3,000 r.p.m., and discard the supernatant. Rinse the remaining suspension into the tube and centrifuge. Drain, stir thoroughly with approximately 25 ml. of water (45 to 50°C.), and centrifuge. Wash in this manner five times for raw meat and six times for cooked meat. In order to determine whether the water-soluble proteins have been removed from the collagenous fiber, apply the biuret test to the final washing.

Hydrolysis: Wash the residue into a 125-ml. Erlenmeyer flask (or 250-ml. beaker, if next operation follows immediately) and disperse well, making the total volume approximately 80 ml. Stopper with a cotton plug and autoclave at 15 pounds pressure for two hours. The material thus adequately protected in sterile condition may be held overnight in the refrigerator.

Pour the supernatant liquid into 40-ml. centrifuge tubes and centrifuge for three minutes (3,000 r.p.m.). Rinse the residue into the centrifuge tube; centrifuge. Mix the residue with approximately 25 ml. of boiling water and place in a hot water bath for two minutes, stirring the mixture with a glass rod. Centrifuge, and repeat the washing five times. Combine all the supernatants in the Kjeldahl flask. Use entire filtrate for the collagen nitrogen determination. Determine total nitrogen on samples of approximately two grams of the original material. Express collagen nitrogen as per cent of total nitrogen.

When the cooked samples were centrifuged following the autoclaving, it was found advisable to drain the supernatant liquid through a paper filter into the Kjeldahl flask. The residue of the cooked sample did not pack firmly enough in the centrifuge tube to prevent the escape of solid material. The use of filters is an added safeguard also with raw samples.

CONCLUSION

A method using the centrifuge and Waring Blendor was developed for the separation of collagen from other meat proteins. This method was found to give reproducible results when applied to raw beef, but was not so precise when applied to cooked beef. The method was compared with the Mitchell method and a revised method by Bell. Data obtained in this laboratory showed the Bell and centrifuge methods gave higher collagen nitrogen results than the Mitchell method, but the discrepancy was not so great as reported by Bell. Results by the centrifuge method tended to run somewhat higher than those obtained by the Bell method.

The chief advantage of the centrifuge method is its adaptability to processing a large number of samples and its great saving of time and tedious manual operation.

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STABILITY OF RIBOFLAVIN IN EGGS TO COOKING AND TO LIGHT[1,2]

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The riboflavin value of raw eggs is known to vary greatly with conditions under which they are produced, the potency being influenced greatly by the level of riboflavin in the hen's diet. Other variations in the riboflavin content of eggs may be due to differences between hens, reported by Jackson, Drake, Slinger, Evans, and Pocock (1946), to day-to-day variation from the same hen, or to seasonal changes as reported by Stamberg and Peterson (1946).

Few investigators have assayed the loss of riboflavin in eggs during cooking. Cheldelin and Williams (1942) reported the cooking losses of riboflavin in scrambled eggs as 35 per cent; Cheldelin, Woods, and Williams (1943) as 22 and 48 per cent, when eggs were scrambled for 10 or 15 minutes in an uncovered pan. They attributed this loss to the effect of light upon the riboflavin in the samples, because when the eggs were scrambled for five minutes in a covered pan, a 100-per cent recovery of the original riboflavin was obtained. Stamberg and Peterson (1946) used individual, consecutive eggs from the same hens and included a comparison of the riboflavin content of raw eggs with that of eggs which were boiled (five per cent loss), fried (7.5 per cent loss), poached (16.5 per cent loss), and scrambled (1.5 per cent loss).

It was the purpose of this study to investigate further the losses of riboflavin in egg cookery and to differentiate the destruction due to moderate kitchen lighting from that due to the heating process involved.

EXPERIMENTAL PROCEDURE

Assay Method: The riboflavin assays were made by the microbiological method of Snell and Strong (1939). In order to eliminate interferring substances in the eggs which stimulated the growth of the microorganism, *Lactobacillus casei*, the acid hydrolysis of the samples was followed by neutralization to from pH 6.6 to 6.8 and blending in a Waring Blendor with chloroform (10 ml. to 200 ml. diluted hydrolyzed egg sample) for half a minute, as suggested by Peterson, Dearstyne, Comstock, and Weldon (1945). After standing 30 minutes in the dark, and filtration of the sample through Whatman No. 40 filter paper, the emulsion broke and a clear extract was obtained for assay.

For the series dealing with irradiation of raw egg, yolk or whites, or of milk, neutralization to pH 4.5 was followed by filtering and then concontinued neutralization to from pH 6.6 to 6.8 to give clear extracts.

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Preparation of Samples: Because preliminary results showed considerable variation in the riboflavin content of individual eggs, a mixture of four dozen eggs was used for a sample. For all the series on boiled, fried, poached, or scrambled eggs, custards, angel food cakes, or soft meringues, the eggs were obtained from the Department of Poultry Husbandry and produced by hens on a ration [3] which contained sufficient riboflavin for good production and hatchability of eggs.

Recipes and ingredients, as well as temperature, time, and manipulative procedures during cooking were carefully standardized. Details of cooking procedures and preparation of the samples for assay have been described fully elsewhere, Anderson (1947). Briefly, for scrambled eggs, 200 gm. were heated with one gm. corn oil in an iron skillet at low heat for seven minutes, with uniform stirring; for fried eggs, two eggs at a time were fried with one gm. corn oil on low heat for five minutes; for poached eggs, two at a time were cooked for five minutes in two cups of water at 90°C.(194°F.). Changes in weight during cooking were recorded. All values reported here have been corrected for those changes. In each case, 48 eggs were combined in a Waring Blendor for one sample and an aliquot taken for assay.

One of the most important precautions taken during the cooking and assays was the control of light. A dark kitchen (0 foot candles on the light meter) was used for all assay procedures as well as for the cooking of one sample of eggs in each series. The only visible light was that which came from an adjoining room and sufficed to make the manipulations possible. The effect of moderate kitchen lighting was studied by the use of an incandescent or fluorescent light. In each case, the light furnished gave a reading of 30 foot candles at the cooking surface. In some series, the eggs were prepared in the sunlight (400 foot candles) which came through a large window nearby. No attempt was made to study riboflavin destruction due to cooking in direct sunshine, since we considered this seldom occurs under ordinary kitchen conditions.

Treatment of Samples for Study on Photolysis: Samples of 25 gm. each from a mix of 36 whites or yolks were placed in 10-cm. Petri dishes and irradiated, covered. During treatment under electric light, the dishes were supported so that a current of air from a fan could be directed below, as well as over the samples, in order to minimize the heating effect of the lamp. Light from an incandescent electric light of 200 foot-candle potency or sunlight of 400 foot-candle potency was applied for three or six hours. For comparison, samples of pasteurized milk were exposed under the same conditions for one and one-half or three hours in electric light, or one-half or one hour in sunlight. Thus, a very rigorous light treatment

[3] Poultry ration L3:

	pct.		pct.
Ground Yellow Corn	30.0	Limestone Grit	1.5
Ground Wheat	30.0	Bone Meal	2.0
Ground Oats	15.0	Salt	0.5
Dehyd. Alfalfa Leaf Meal	5.0	Fish Oil 85D	0.5
Meat Scrap	4.0	Riboflavin (mg./lb.)	1.0
Soybean Oil	20.0	Fish Solubles (25 per cent solids)	5.0
Granite Grit	1.5		

was given milk or raw egg yolks or whites without the factor of heating or ultraviolet irradiation, except as it was not screened out by the pyrex cover of the Petri dish.

Statistical Analysis: Analyses of variance, Snedecor (1940), were made on the data for the scrambled, fried, and poached eggs.

RESULTS AND DISCUSSION

The effect of moderate amounts of visible light upon the riboflavin content of eggs during cooking, as well as the effect of the heating, are shown (Tables 1 and 2). Data show that about 10 per cent of the original

TABLE 1

Effect of Light and of Cooking on Riboflavin of Scrambled Eggs

Series No.	Raw eggs	Scrambled in dark	Scrambled in electric light[1]	Scrambled in fluorescent light[1]	Scrambled in sunlight[2]
	μg./gm.	μg./gm.	μg./gm.	μg./gm.	μg./gm.
1	2.52	2.38	2.33	2.32	.....
2	2.55	2.37	2.34	2.30	2.25
3	2.49	2.19	2.18	2.17	2.15
4	2.44	2.21	2.19	2.18	.....
5	2.54	2.29	2.27	2.25	2.18
6	2.50	2.24	2.22	2.24	.....
Average	2.50	2.28	2.26	2.24	2.20
Per cent loss	.....	8.80	9.60	10.40	13.00

[1] 30 foot candles. [2] 4 0 foot candles, through glass.

TABLE 2

Effect of Light and of Cooking on Stability of Riboflavin in Fried and Poached Eggs

Series No.	Raw eggs	Fried in dark	Fried in sunlight[1]	Series No.	Raw eggs	Poached eggs
	μg./gm.	μg./gm.	μg./gm.		μg./gm.	μg./gm.
7	2.80	2.34	2.42	10	2.63	2.28
8	2.82	2.37	2.37	11	2.68	2.33
9	2.75	2.25	2.27	12	2.70	2.36
Average	2.79	2.31	2.35	.....	2.67	2.32
Per cent loss	.....	17.20	15.80	.....	.....	13.10

[1] 400 foot candles, through glass.

riboflavin of eggs was destroyed during scrambling, whether in the dark, incandescent or fluorescent light; 13 per cent was destroyed in bright sunlight. About 16 per cent of the riboflavin was lost in frying, and 13 per cent in poaching. Statistically, the losses obtained show no significant differences due to variation in the kitchen lighting, except for the three samples scrambled in sunlight. Heating itself was the more destructive agent in the cooking procedure. In every case, there was a significant difference between raw and cooked.

One sample of 48 eggs hard-cooked for 20 minutes at 90°C. was found to show 1.1 per cent loss in riboflavin. A similar small loss had been obtained previously, Anderson (1947), using the fluorometric method of assay for eggs held in boiling water 10 minutes. What protection is

provided other than the darkness cannot be suggested from the present study.

Effect of cooking upon the riboflavin of custards, angel food cakes, and soft meringue has not been reported in the literature heretofore. Analyses of custards reported (Table 3) show that greater destruction of the riboflavin occurred in baked custards, where the effective agent was heat only, than in stirred custards prepared in a glass double boiler under light treatment. The proportion of the destroyed riboflavin due to milk or egg source could not be determined.

TABLE 3

Effect of Light and of Cooking on Riboflavin of Custards

Series No.	Raw egg	Riboflavin in 1 gram custard mixture due to egg	Total riboflavin in uncooked custard mixture	Baked custard[1]	Stirred custard[2]
	μg./gm.	*μg./gm.*	*μg./gm.*	*μg./gm.*	*μg./gm.*
14	2.54	0.85	1.70	1.44	1.80
15	2.52	0.85	1.22	1.12	1.12
16	2.55	0.85	1.22	1.12	1.13
17	2.48	0.83	2.24	1.85	1.84
18	2.44	0.83	1.11	0.91	0.93
19	2.54	0.85	1.22	1.07	1.04
Average	2.51	0.85	1.45	1.25	1.31
Per cent loss	.....	.....	.....	13.80	9.70

[1] Baked in a pan of water in a 350° F. oven to a temperature of 84° C. [2] Cooked in glass double boiler to 82° C. (30 foot candles of light).

In soft meringue, where a large surface was exposed for evaporation, there was an excessive loss of moisture (30 per cent) and an accompanying 23.4 per cent loss of the original riboflavin. Angel food cake appeared to have a very low concentration of riboflavin, even before baking, perhaps because of dilution with high concentrations of sugar. Approximately 10 per cent of the original riboflavin (some present in egg white and some in flour) in angel food cakes was destroyed, regardless of the presence or absence of light during the mixing process. These determinations indicate that an average serving (40 grams) of angel food cake would contain only 0.025 milligram of riboflavin.

The marked stability to light of the form of riboflavin present in eggs is shown further (Table 4). Raw egg yolks or whites exposed in a thin layer (25 gm. in 10-cm. Petri dish) to ordinary electric light or to sunlight are in sharp contrast to the samples of milk exposed at the same time, but for shorter periods. The one comparable period of three hours in the electric light showed no loss of riboflavin in yolks, up to eight per cent in whites, but about 95 per cent destruction in the milk samples. The riboflavin of milk has been shown to be present chiefly in the free rather than the bound forms, György, Kuhn, and Wagner-Jauregg (1934), Kuhn and Katschmitt (1935). It has been repeatedly observed that riboflavin in solution and the riboflavin of milk are easily destroyed by visible light treatment and even more so by ultraviolet irradiation. In the cooking phase of this study, light from electric sources and sunlight through window-

glass can be assumed to give no wavelengths of 3000Å or less. Pyrex glass of which the Petri dishes were made, however, transmits five per cent of the irradiation of 3000Å wavelength. Thus, sunlight through the cover of the Petri dish may be expected to be slightly more destructive to riboflavin since it transmits some ultraviolet waves.

TABLE 4

Comparative Stability to Light of Riboflavin of Yolks and Whites of Eggs

	Irradiation	Sample 1 concentration	Loss	Sample 2 concentration	Loss
		μg./gm.	*pct.*	*μg./gm.*	*pct.*
Raw egg yolk	None	3.88	.....	3.59	.....
	Electric light[1] —3 hr.	3.85	0.8	3.67	+2.2
	Electric light[1] —6 hr.	3.80	2.1	3.29	8.3
	Sunlight through glass[2] —3 hr.	3.62	6.7	3.52	2.0
	Sunlight through glass[2] —6 hr.	3.62	6.7	3.48	3.1
Raw egg white	None	2.76	.....	2.99	.....
	Electric light[1] —3 hr.	2.72	1.4	2.74	8.0
	Electric light[1] —6 hr.	2.61	5.4	2.75	8.1
	Sunlight through glass[2] —3 hr.	2.48	10.1	2.45	18.0
	Sunlight through glass[2] —6 hr.	2.01	27.2	2.10	29.8
Milk pasteurized	None	1.72	.....	1.90	.....
	Electric light[1] —1½ hr.	0.25	85.5	0.22	88.4
	Electric light[1] —3 hr.	0.12	93.0	0.08	95.8
	Sunlight through glass[2] —½ hr.	0.14	91.9	0.19	90.0
	Sunlight through glass[2] —1 hr.	0.11	93.8	0.07	96.3

[1] 200 foot candles. [2] 400 foot candles.

The variation of riboflavin destruction (Table 4) due to electric light on the yolks, as contrasted to the whites, is within the limits of experimental error of the analytical method. For the sunlight treatment, however, higher losses occurred in the whites than in the yolks, particularly for the six-hour period. Whether this difference is due entirely to some variation in condition such as the higher pH value of whites, or to a difference in the proportion of the more and the less photo-stable forms of riboflavin, cannot be determined from these data. Two other series of samples were analyzed for riboflavin but were not neutralized in the same manner and the yolk extracts were not clear. However, the general trend was the same as for the analyses reported in detail. Thus, the riboflavin assays of eggs at room temperature confirm the fact shown in the cooking series, that the form of riboflavin in eggs is remarkably stable to ordinary indoor light.

Numerous factors might be listed as influencing the final potency of riboflavin in eggs as eaten. Time and intensity of heating, the form in which riboflavin occurs, the wavelength, potency of light and the surface exposed, the pH, and other possible characteristics of the egg, are all factors to be considered. This study presents data which indicate that ordinary lighting during cooking is not a major factor in the destruction of riboflavin in eggs and that the form of riboflavin in eggs is remarkably photostable.

SUMMARY AND CONCLUSIONS

The riboflavin of egg yolk or white was remarkably photostable when exposed to electric light (200 foot candles) or sunlight through glass (400 foot candles) at room temperatures for long periods.

Eggs were prepared for the table under carefully controlled conditions, including the potency of indoor lighting (30 foot candles for electric lighting or 400 foot candles through windowglass for sunlight). There was no statistically significant difference in the destruction during cooking whether in the dark, in incandescent or fluorescent light. Scrambled eggs prepared in sunlight (400 foot candles through glass) showed a significantly greater loss than those prepared in the dark; this was not true for fried eggs.

Hard-cooked eggs showed no appreciable loss of their original riboflavin; scrambling, except in strong sunlight, caused about 10 per cent destruction; frying resulted in about 16 per cent destruction; and poaching destroyed about 13 per cent riboflavin.

When eggs were combined with other ingredients into food products, it was found that stirred or baked custards lost from 10 to 14 per cent of their original ribaflavin and that the cooking heat during baking was more destructive to the riboflavin of custards than ordinary electric light and heating together were to stirred custards prepared in a glass double boiler. Angel food cakes contained only a small amount of riboflavin, about 10 per cent of which was destroyed during the baking process whether the cake batter was mixed in the dark or in the light.

The baking of soft meringue resulted in a large loss of riboflavin. As much as 24 per cent of the original riboflavin in these samples of egg white was destroyed when they were baked as soft meringue.

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SOLUBILITY OF OXYGEN IN SOLUTIONS OF VARIOUS SUGARS

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The absorption of oxygen from the air during the preparation and use of sugar solutions in the frozen fruit industry contributes to the oxidative deterioration of frozen fruits. There is little data available in the literature on the solubility of oxygen in sugar solutions and the effect of concentration, type of sugar, and method of preparation on oxygen content. Markham and Kobe (1941) in their review of the solubility of gases in liquids cite only two papers: that of Müller (1912), who determined the solubility of pure oxygen in solutions of sucrose, dextrose, glycerol, and chloral hydrate by a physical saturation method; and that of MacArthur (1916), who determined the solubility of the oxygen of the air in sucrose solutions by a modified Winkler titrimetric procedure. Markham and Kobe (1941) rated both data as being only moderately reliable. Müller (1912) measured the Bunsen absorption coefficient at a total pressure of one atmosphere and at temperatures varying from 15.6 to 17.2°C.(61 to 62°F.) for sucrose solutions, 19.9 to 21.7°C.(68 to 72°F.) for glucose solutions, and 12.2 to 14.6°C.(54 to 59°F.) for glycerol solutions. Müller's data was recalculated by Smith (1928) as the true Bunsen coefficient at 15°C. for glycerol and 20°C. for glucose and sucrose. MacArthur (1916) saturated the sugar solutions with oxygen by shaking several times for about four days in a tall cylinder placed in a thermostat at 25°C.; their oxygen content was then determined by use of an alkaline manganous chloride solution. He noted that the sugar was slowly oxidized by the dissolved oxygen, but found that fairly constant results could be obtained if the solutions were shaken vigorously immediately before addition of the manganous chloride and alkaline iodide.

Müller's data are plotted as α vs. M_c (Fig. 1) and as the actual β values observed vs. per cent (Fig. 2). It is interesting that the solubility of oxygen in sucrose solutions decreases more rapidly with molar concentration than in glucose or glycerol solutions, and in solutions of one M or above the solubility of oxygen is greater in glucose than in sucrose solutions. When plotted on a per cent solids content basis, the solubility of oxygen in sucrose solutions at about 16°C. is greater than in glucose solutions at 21°C., up to about 40 per cent, and above that the solubility is less in sucrose solutions even though it was measured at a lower temperature. MacArthur's data (plotted Fig. 3) show a progressive and rapid decrease in solubility of oxygen, with increase in concentration of sucrose. His values are lower than those of Müller, because of the presence of dissolved nitrogen as well as oxygen, and because his measurements taken at a total pressure of one atmosphere were actually equivalent to

a partial pressure of oxygen of less than 0.21 atmosphere. He reported the solubility of oxygen in distilled water at 25°C. and one atmosphere total pressure as 5.78 c.c. per liter. The solubility of air in water at 25°C. is given in the literature as 17.08 c.c., of which 33.82 per cent, or 5.88 c.c., is oxygen, Loomis (1928). The Bunsen coefficient of solubility of oxygen in water is given by Fox (1909) as 29 x 10^{-3} equivalent to 29 c.c. per liter; Smith (1928) prefers 28.22 as the more consistent value, which,

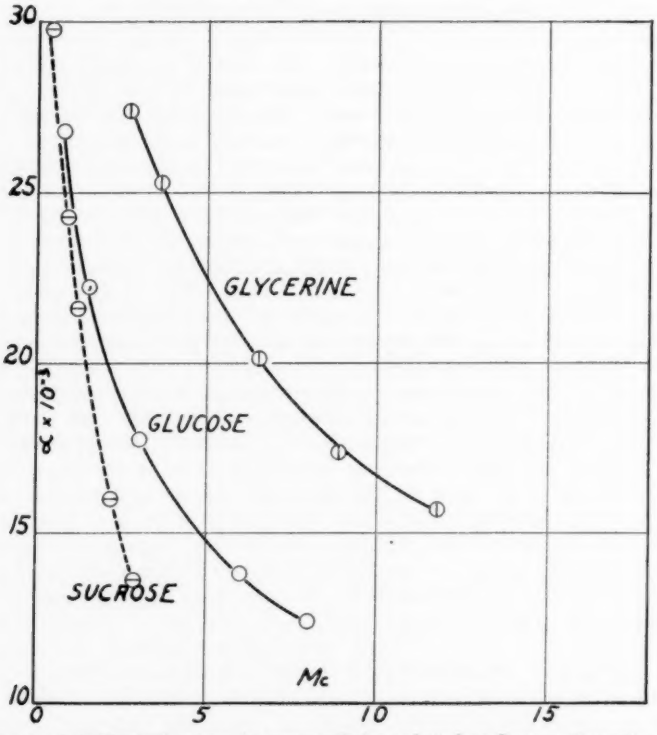

FIG. 1. Solubility of oxygen in sugar solutions plotted as Bunsen absorption coefficient α against molar concentration, from Müller's (1912) data.

neglecting water vapor pressure would be roughly equivalent to a solubility of 5.93 c.c. of oxygen from air containing 0.21 mol. per cent of oxygen.

We have obtained some data on the solubility of oxygen in equilibrium with air atmospheres in sugar solutions prepared from various analytical and commercial sugars and sirups. Since these data were intended to illustrate the effect of type and concentration of sugar, more attention was given to rapid determination of oxygen content that might be encountered under commercial conditions in these sirups, than to an accurate determination of solubility relations. The latter phase of the project is now under investigation.

EXPERIMENTAL PROCEDURE

The sugars or sirups were dissolved in warm 0.01 M KCl solution to minimize errors due to migration currents, and cooled to room temperature by allowing to stand overnight. The temperatures at which the determinations were made varied from 19 to 24°C.(66 to 75°F.). The maximum of the first oxygen wave was suppressed by addition of 0.1 c.c. of a 0.1 per cent methyl red indicator as recommended by Kolthoff and

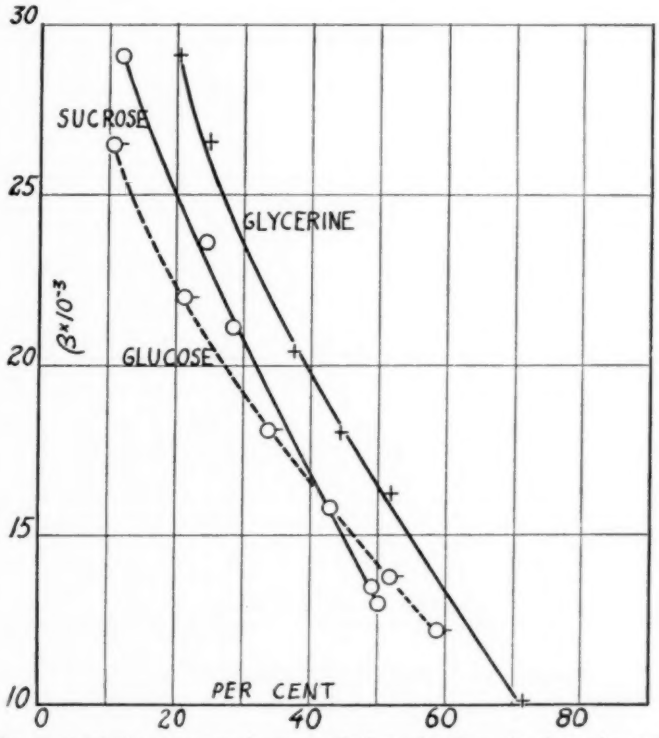

FIG. 2. Solubility of oxygen in sugar solutions plotted as the absorption coefficient at one atmosphere total pressure against concentration in per cent by weight from Müller's (1912) data.

Lingane (1941) and Kolthoff and Miller (1941). The solutions were then aerated for two hours with a sintered glass bubbler and after aeration allowed to stand in open beakers exposed to air for 20 minutes. It was found that after one and one-half to two hours' aeration the solutions became fully saturated with oxygen under our experimental conditions. Since there was a tendency for the solutions to be supersaturated, they were allowed to stand so that the dissolved oxygen would come to equilibrium with air. It was found that the amount of dissolved oxygen slowly

decreased during the first 15 minutes after aeration, then remained constant. Oxygen content of the resulting solutions was determined by the manually operated Fisher Scientific Company Electropode. The current difference for the second oxygen wave between 0.8 and 1.4 volts was taken as a measure of the oxygen content. The polarographic method has been successfully used for the determination of oxygen in a variety of biological materials: Petering and Daniels (1938), Baumberger (1939), Hartman and Garrett (1942), Lindquist (1947), and Lewis and McKenzie (1947). The polarographic data were calibrated against oxygen content as determined chemically by a modified Winkler procedure, A.O.A.C. (1945), as

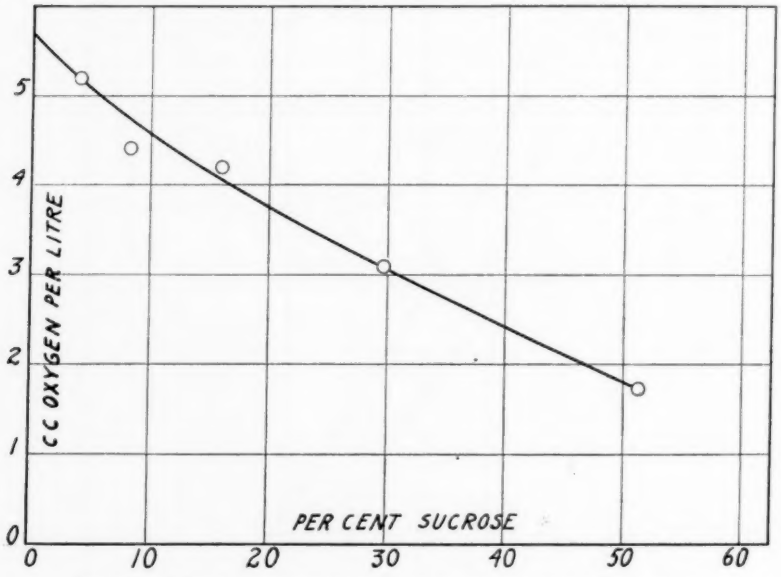

FIG. 3. Solubility of oxygen from air in sucrose solutions from MacArthur's (1916) data.

well as from solubility data in the literature. Typical comparisons are given below.

For air-saturated 0.01 M KCl at 18°C. the chemical analysis gave 6.60 c.c. of O_2 at S.T.P. per liter and in 30 per cent sucrose solution at 18°C. it was 3.61 c.c. The current difference obtained polarographically was 26.0 for 0.01 M KCl solution and 14.8 for the sucrose solution. The oxygen content of the sucrose solution calculated polarographically was $\frac{14.8}{26.0} \times 6.60 = 3.76$.

In another test the chemical analyses for air-saturated 0.01 M KCl and 30 per cent sucrose solutions at 21°C. were 6.36 and 3.54, respectively; current differences were 26.8 and 14.8, respectively. Oxygen content of

the sucrose solution at 21°C., calculated from the polarographic data, was $\frac{14.8}{26.8} \times 6.36 = 3.51$. These data agree fairly well with the chemical determination, but are somewhat higher than the values reported by MacArthur (1916).

TABLE 1

Oxygen Absorbed From Air by Sugar Solutions[1]

Conc. on solids basis	Dextrose	Levulose	Sucrose	Cane sugar	Cerelose	Baker's glucose	Sweetose	Puritose	Invert
pct.									
6.5	.....	.....	.....	5.54	.....	.....	.....	.....	4.90
7.6	.....	.....	.....	.....	.....	.....	4.96	.....	.....
8.4	.....	.....	.....	.....	.....	4.22	.....	3.70	.....
10.0	5.58	4.76	5.31	.....	5.56	.....	.....	.....	4.36
13.0	.....	.....	.....	4.58	.....	.....	.....	.....	4.02
15.2	.....	.....	.....	.....	.....	.....	2.84	.....	.....
16.8	.....	.....	.....	.....	.....	.....	.....	3.42	.....
17.0	.....	.....	.....	.....	.....	3.36	.....	.....	.....
19.5	.....	.....	.....	.....	.....	.....	.....	.....	2.89
20.0	4.27	.....	4.30	.....	3.70	.....	.....	.....	.....
22.0	.....	.....	...	.....	.....	.....	2.58	.....	.....
25.2	.....	.....	.....	.....	.....	2.97	.....	2.72	.....
26.0	.....	.....	.....	3.08	.....	.....	.....	.....	2.34
30.0	.....	3.10	3.43	.....	3.00	.....	.....	.....	2.00
30.5	.....	.....	.....	.....	.....	.....	2.07	.....	.....
33.7	.....	.....	.....	2.20	.....	.....	.....	2.04	.....
38.0	.....	.....	.....	.....	.....	.....	2.04	.....	.....
39.0	.....	.....	.....	.....	.....	.....	.....	.....	1.05
40.0	2.18	.....	2.56	1.31	2.38	.....	.....	.....	.....
42.2	.....	.....	.....	.....	.....	2.64	.....	.....	.....
45.7	.....	.....	.....	.....	.....	.....	1.41	.....	.....
50.0	.....	.....	.....	.....	2.04	.....	.....	.....	.....
50.7	.....	.....	.....	.....	.....	1.20	.....	.....	.....
60.0	1.48	.....	0.71	.....	1.52	.....	.....	.....	.....
Temp. °C.	19	23	21-23	24	23	21	22.5	24.5	24

[1] Oxygen content in c.c. at S.T.P. per liter solution.

OXYGEN ABSORBED FROM AIR BY SUGAR SOLUTIONS

The amount of oxygen absorbed by aeration of sugar solutions of various concentrations prepared from different sugars and sirups is shown (Table 1 and Fig. 4). The sugars used in order given were C. P. dextrose, levulose, and sucrose; a commercial cane sugar solution of 66.5 per cent solids content; commercial corn sugar; baker's glucose (low conversion corn sirup) of 84.3 per cent solids content; an enzyme converted corn sirup of 76.2 per cent solids content; a high conversion corn sirup of 84 per cent solids content; and a 90 per cent inverted sucrose sirup of 65 per cent solids content. On the basis of these data the solubility of oxygen in the sugar solutions at a 20 per cent solids content is greatest in sucrose solutions and least in "sweetose" solutions, the order of decreasing solubility being sucrose, dextrose, levulose, cerelose, baker's glucose, puritose, invert, and sweetose. The solubility ranges from 4.3 in sucrose solutions to about 2.5 in sweetose solutions. At 40 per cent solids

content the sugars in order of decreasing oxygen content were sucrose, cerelose, dextrose, baker's glucose, puritose, sweetose, and invert. In general, the difference between the sugars is more profound at lower than at higher concentrations.

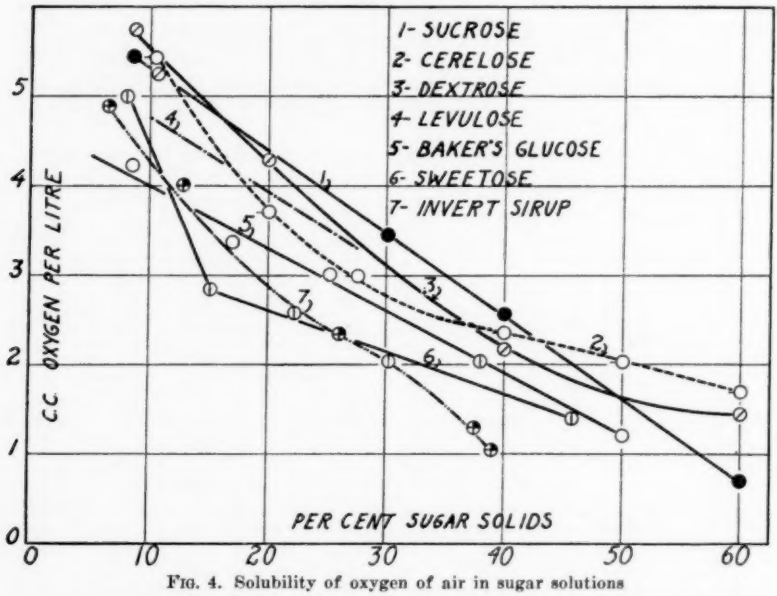

FIG. 4. Solubility of oxygen of air in sugar solutions

1—sucrose; 2—cerelose; 3—dextrose; 4—levulose; 5—baker's glucose sirup; 6—"sweetose" corn sirup; 7—90 per cent invert sirup.

The effect of method of preparing sirups is shown (Table 2). In one case the sirups were prepared by dissolving the necessary amount of granulated sugar in water at 20°C. with vigorous stirring; in the other case the water was brought to boiling and the sugar dissolved with a minimum of stirring, then allowed to cool to 20°C. in uncovered beakers. There was little difference under these conditions at lower densities, but the percentage difference progressively increased with increase in density. Some diffusion of oxygen undoubtedly occurred during cooling of the hot sirup, but this would also occur under commercial practice. These data indicate but little difference between the two methods. Under commercial practice, however, occluded oxygen as well as dissolved oxygen would be incorporated in the sirup.

TABLE 2

Effect of Method of Dissolving Granulated Cane Sugar on Oxygen Content

Sirup density, ° Balling	20	30	40	50
Solution in cold, with stirring	3.15	2.27	1.68	1.08
Solution in hot	3.05	2.19	1.42	0.78

SUMMARY AND CONCLUSIONS

The oxygen content of solutions of various sugars and sirups in equilibrium with air was determined by a polarographic procedure. Noticeable differences were found between solutions of the same solids content, and the ability to dissolve and retain oxygen was not related to molecular concentrations and hence the osmotic pressure. The nature of this specific effect of sugars was not determined.

The method of preparation of sugar solutions influenced their oxygen content. More oxygen was present in solutions prepared by stirring at room temperature, than in solutions prepared by heating, particularly at higher densities.

ACKNOWLEDGMENT

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EFFECT OF SUGARS ON CONVERSION OF DEHYDRO ASCORBIC ACID

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Dehydro-l-ascorbic acid in aqueous solutions undergoes mutarotation which is generally assumed to be due to the gradual opening of its lactone ring with the formation of 2:3-diketo-l-gulonic acid. Penney and Zilva (1943a) have developed a colorimetric procedure for determination of the gulonic acid based on the formation of a 2:4-dinitro-phenylhydrazone, which in alkaline solution gives an intensely red-colored pigment whose concentration can be readily determined by a photoelectric colorimeter with a green color filter. They have used this procedure in the study of the chemical behavior of dehydro ascorbic acid *in vitro* and *in vivo* (1943b). Formation of gulonic acid from dehydro ascorbic acid was found to be a first-order reaction below about pH 4; it is accelerated by increase in temperature and at higher pH values. Thus, Penney and Zilva (1943b) report a 30 per cent conversion in 300 minutes at pH 4 and an 80 per cent conversion in 15 minutes at pH 7.4. In the course of our investigations on the technological properties of sugars, Joslyn (1949) and Joslyn and Miller (1949), it was of interest to determine to what degree hydrolytic conversion of dehydro ascorbic acid into gulonic acid would be influenced by sugars. For this purpose the rate of formation of gulonic acid was investigated in presence of several different sugars at two levels of concentration and the data presented here.

EXPERIMENTAL PROCEDURE

Dehydro ascorbic acid was prepared by dissolving 0.5 gm. Hoffman-La Roche 1 (±) ascorbic acid in a small volume of water, and oxidized by adding an equivalent amount of 0.01 N iodine solution. This was transferred to a 100-ml. volumetric flask and sufficient dilute NaOH added to neutralize the HI formed, then brought to volume. Sugars or sirups used were dissolved in McIlvaine's phosphate-citrate buffer at pH 5 and brought to slightly below 40 ml. the day before they were to be used. Just before preparation of the dehydro ascorbic acid the sugar solutions were brought to 30°C.(86°F.) and held at this temperature. Then 10 c.c. of the freshly prepared 0.5 per cent dehydro ascorbic acid were added and the volume brought to 50 c.c. This addition lowered the temperature to about 28°C.(82°F.) and after mixing, the solution was transferred to a water bath held at 28.2°C. Gulonic acid content was determined periodically by withdrawing a sufficient aliquot to give a reading between 100 and 500 on the Klett-Summerson colorimeter. This aliquot was added to reaction tubes containing one c.c. of dinitrophenylhydrazine reagent in two N HCl (two gm. of the hydrazine per liter) and eight c.c. of water at bath temperature. The mixture was incubated for one hour at 28.2°C.,

transferred to a 25-c.c. volumetric flask, four c.c. of one N NaOH added, and made to volume. The colorimeter reading was taken one minute after adding NaOH and two minutes after removing the tube from the water bath. A control was prepared at the same time and in the same way. The colorimeter was calibrated against freshly prepared dehydro ascorbic acid (0 per cent conversion) and an 18-day-old solution (100 per cent conversion) as described by Penney and Zilva (1943a).

TABLE 1

Per Cent Conversion of Dehydro Ascorbic Acid in Presence of Sugars

In 20 per cent sugar solutions

Time	Control	Dextrose	Fructose	Invert
hr.	*pct.*	*pct.*	*pct.*	*pct.*
0	00.0	00.0	00.0	00.0
3	16.3	13.5	14.3	10.3
5	19.2	17.0	20.5	17.0
8	30.0	23.0	26.0	24.0
9	.....	.....	27.4	26.8
10	34.8	25.7	.....	.....
12	38.2	29.8	34.0	32.0
24	56.1	43.5	51.6	50.0
27	.....	46.2	53.5	51.6
30	58.0	48.0	.....	53.4

In 40 per cent sugar solutions

Time	Control	Dextrose	Sucrose	Lactose	Maltose	Baker's glucose	Puritose	Sweetose
hr.	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*
0	00.0	00.0	00.0	00.0	00.0	00.0	00.0	00.0
3.5	15.5	10.0	17.3	.....	.....	.....	.....	.....
4.0	.....	.....	.....	07.5	14.1	20.0	13.0	18.0
4.5	15.8	.....	.....	.....	.....	.....	.....	.....
5.0	21.0	13.0	22.5	.....	19.0	.....	.....	.....
6.0	.....	.....	24.0	.....	.....	.....	.....	.....
6.5	22.3	.....	.....	.....	.....	.....	.....	.....
8.0	29.2	16.7	30.0	14.3	23.0	.....	.....	.....
8.5	28.5	.....	.....	.....	.....	.....	.....	.....
9.0	.....	.....	28.4	.....	.....	.....	.....	.....
9.25	33.0	.....	.....	.....	.....	.....	.....	.....
10.0	.....	.....	32.4	.....	.....	31.0	25.0	32.0
16.0	.....	.....	.....	.....	.....	37.5	33.0	40.6
20.0	50.0	25.5	.....	37.8	37.8	.....	.....	.....
24.0	55.0	33.6	47.5	50.8	40.0	42.0	35.8	45.2
30.0	58.0	36.8	50.0	53.0	43.0	43.5	37.8	46.3

Sugars used were C. P. dextrose, fructose, sucrose, lactose and maltose and invert sirup, baker's glucose (low conversion corn sirup), puritose and sweetose (high conversion corn sirups). The solutions were brought to 20 per cent and 40 per cent sugar or sirup solids concentration. Data on rate of formation of 2:3-ketogulonic acid from a 0.1 per cent dehydro ascorbic acid solution with and without added sugars at pH 5 and at 28.2°C. are given (Table 1). Actual data are given, since the reaction was not first-order except for a short initial period (see Figs. 1 to 3), nor of second- or zero-order.

DISCUSSION

The data indicate that in general the sugars tested have a protective effect on the hydrolysis of dehydro ascorbic acid. At a level of 20 per cent, dextrose has a greater effect than fructose or invert sugar, except initially during the first five hours, when invert sugar is somewhat more

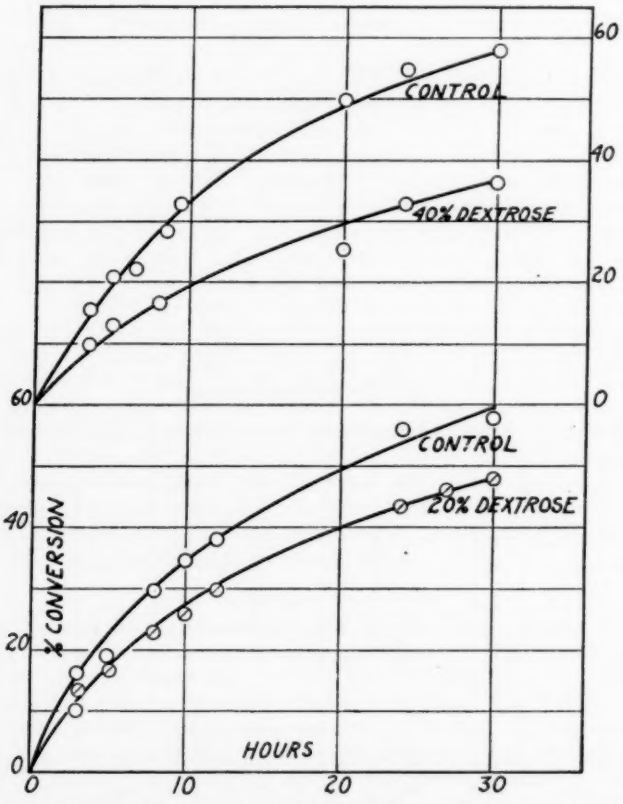

FIG. 1. Effect of dextrose on rate of conversion of dehydro ascorbic acid into 2,3-diketogulonic acid at pH 5 and 28°C. (linear plot).

protective. Fructose is least protective and initially has no protective effect, but after 24 hours is equivalent to invert sugar. At a level of 40 per cent, dextrose again is the most protective, being closely followed by puritose. Sucrose offers but little protection and initially even exerts a catalytic effect. At the higher level of sugar content, and particularly at longer reaction times, discoloration of the sugar-dehydro ascorbic acid solutions tends to make determination difficult and the data less reliable.

SUMMARY

Rate of conversion of dehydro ascorbic acid into gulonic acid was determined in presence of two concentrations of various sugars. Dextrose was found to inhibit this reaction, the extent of inhibition increasing with increase in dextrose concentration. At concentration of 40 per cent the sugars in order of greatest protection were dextrose, puritose, maltose, baker's glucose, sweetose, sucrose, and lactose, after 24 hours.

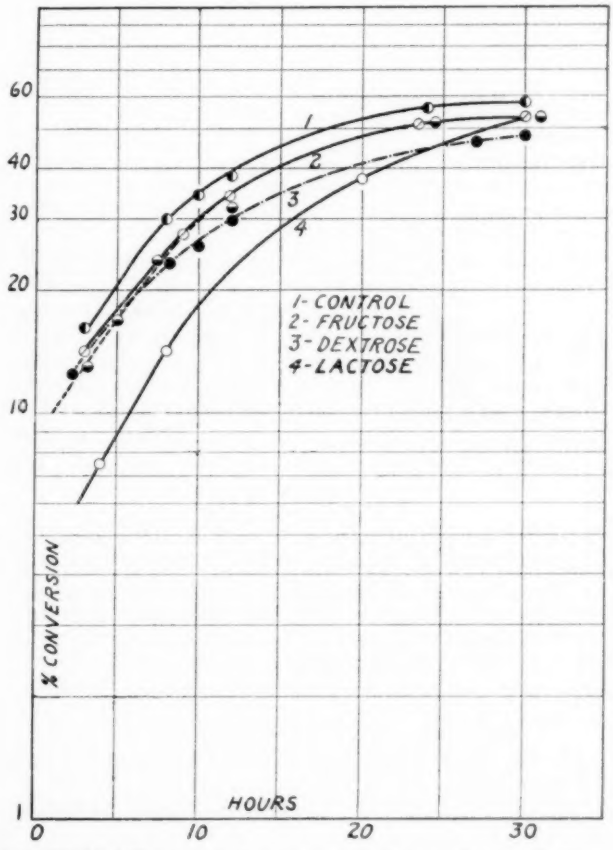

FIG. 2. Effect of 20 per cent of sugar on rate of conversion of dehydro ascorbic acid into 2,3-diketogulonic acid at pH 5 and 28°C. (semi-log plot).

ACKNOWLEDGMENT

These investigations were aided by a research grant from the Sugar Research Foundation.

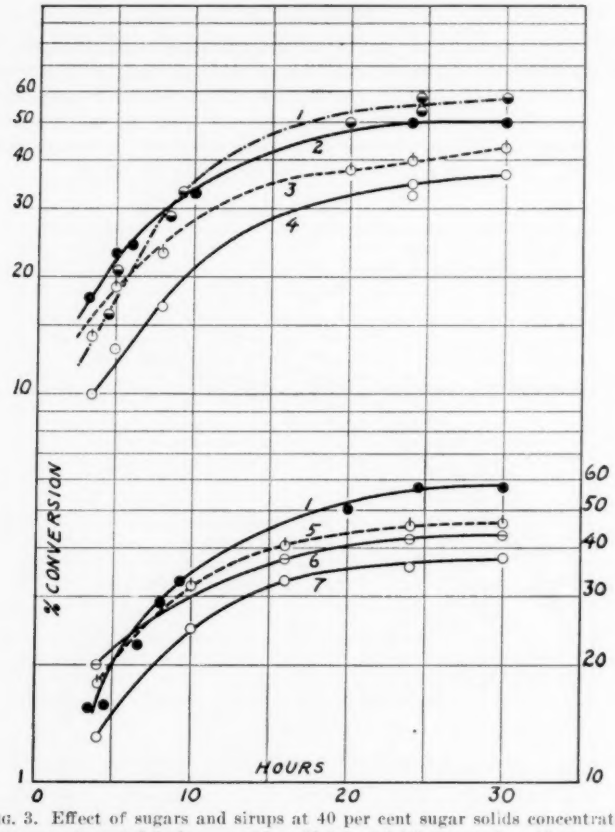

FIG. 3. Effect of sugars and sirups at 40 per cent sugar solids concentration on rate of conversion of dehydro ascorbic acid into 2,3-diketogulonic acid at pH 5 and 28°C. (semi-log plot). 1—control; 2—sucrose; 3—maltose; 4—dextrose; 5—sweetose corn sirup; 6—glucose corn sirup; 7-puritose corn sirup.

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COOKING LOSSES, TENDERNESS, PALATABILITY, AND THIAMINE AND RIBOFLAVIN CONTENT OF BEEF AS AFFECTED BY ROASTING, PRESSURE SAUCEPAN COOKING, AND BROILING [1]

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Investigations during recent years have shown that time, temperature, and type of cooking rank high among the factors influencing tenderness, palatability, and vitamin retention in cooked meat. The current use of the pressure saucepan in meat cookery arouses interest in the effect on these three factors of cooking under pressure. This study was undertaken to investigate the effect of pressure saucepan cookery as compared with the effect of oven roasting on the tenderness, palatability, and thiamine and riboflavin retention of meat. Twelve paired top round beef roasts were used; one of each pair was analyzed raw and the other either cooked in the pressure saucepan or roasted in the oven.

Subsequently, the amounts of thiamine and riboflavin present in the thawed frozen ground meat after four and eight months of freezer storage and after pan and oven broiling were determined. For this secondary phase of the study, meat prepared by grinding the raw roasts had been frozen. Moisture, ether extract, and protein determinations were made on the raw, thawed frozen, and cooked meat.

REVIEW OF LITERATURE

Although some work has been done on the effect of different cooking methods, on cooking losses, and on the retention of thiamine and riboflavin in beef, no reports were found in the literature dealing with the comparative tenderness and palatability of top rounds of beef which had been cooked in the pressure saucepan or roasted in the oven.

Cover (1943) found a total cooking loss of 32.6 per cent when beef round was roasted. Meat which had been stewed in the pressure saucepan was reported to have a cooking loss of 40.7 per cent by Cover, Dilsaver, and Hayes (1947). A smaller loss of 34 per cent was found by Tucker, Hinman, and Halliday (1946).

Booher and Hartzler (1939) found that top round of beef contained 1.14 micrograms of thiamine per gram. Tucker *et al.* (1946) reported that beef round contained 1.13 micrograms of thiamine per gram and that 65 per cent of this amount was retained when the meat was braised in the pressure saucepan. When Cover and Dilsaver (1947) stewed beef in the pressure saucepan, the cooked meat retained from 43 to 60 per cent

[1] This paper represents a portion of the thesis submitted by Ruth V. Keys to the Graduate School of the University of Illinois in partial fulfillment for the degree of Master of Science.

of the thiamine originally present. In a study carried on by Cover, McLaren, and Pearson (1944), thiamine retention in beef which had been oven roasted ranged from 69 to 75 per cent.

Top round of beef contains 2.14 to 2.22 micrograms of riboflavin per gram as reported by Munsell (1942) who used the rat growth method of assay. Tucker *et al.* (1946) employed a fluorometric method and found that beef round had 1.83 micrograms of riboflavin per gram. The latter authors also reported that 81 per cent of the riboflavin was retained in the meat when beef was braised in the pressure saucepan. This value is within the range of 77 to 83 per cent found by Cover *et al.* (1944) when beef was roasted in the oven. Cover and Dilsaver (1947a) report a 66 to 68 per cent riboflavin retention in beef stewed in the pressure saucepan.

EXPERIMENTAL PROCEDURE

Source and Description of Meat: Twelve paired top round beef roasts of choice and prime quality were obtained from the Animal Science Department. The animals had been fed on a corn diet in a dry lot and were butchered from May 20 to July 8, 1947. The carcasses were cut two days after slaughter. In each instance the first two and one-half inches of top round were used to give roasts weighing approximately five pounds. The roasts were then wrapped in two layers of pliofilm and finally in a sheet of locker paper and aged for 12 days at 0 to 3.3°C.(32 to 38°F.). At the end of these periods two pairs of roasts were brought to the food research laboratory, where cooking and sampling were done on the same day. The four roasts were weighed and measured, after which they were judged subjectively for quality, according to the form given by the National Cooperative Project Supplement of the United States Department of Agriculture (1933). One member of each pair, or two roasts, were ground and sampled for analysis of the raw meat. Of the remaining two roasts one was cooked in the pressure saucepan, the other roasted in the oven. The experiment was planned so that six of the cuts from the right side were analyzed raw. Three of the corresponding left cuts were oven roasted, the remaining three cooked in the pressure saucepan. With the other six pairs the process was reversed, i.e., the left cuts were analyzed raw and the right cuts were cooked.

Methods of Cooking: The cut to be roasted was placed on a flat aluminum rack in a long, shallow pan. The mean temperature of the electric oven used was 148.9°C.(300°F.) and the meat was roasted to an internal temperature of 82°C.(179.6°F.). When the meat was cooked in the pressure saucepan, the roast was put on the rack and three tablespoons of water added. The meat was cooked at 15 pounds pressure, 121.1°C. (250°F.). The flame was turned off when the internal temperature of the meat was 65°C.(149°F.) so that the final temperature reached but did not exceed 82°C.(179.6°F.).

Heat Penetration and Cooking Losses: The oven and the pressure saucepan were fitted with copper and constantan thermocouples connected to a Leeds and Northrup Micromax Recorder. This equipment gave a record of the internal temperature of the meat and of the temperature of the space surrounding the meat every other minute. These temperatures

and time intervals were used to compare the rates of heat penetration. Weighings of the raw and cooked roasts and of the drippings were made to determine the cooking losses.

Tenderness and Palatability: Two cylindrical cores one inch in diameter were taken from each cooked roast, one from the semimembranous muscle, the other from the abductor muscle. These cores were tested for tenderness on a modified Warner-Bratzler shear stress apparatus. Three readings were obtained on each core.

A longitudinal slice was removed from the center of the roast and rated subjectively, using the score card as given by the National Cooperative Project of the United States Department of Agriculture (1927). Five experienced judges scored the meat on the basis of aroma, texture, flavor of fat and lean, and the quality and quantity of the juice.

Preparation for Sampling: The raw and cooked roasts were each cut into one-half- to one-inch cubes and ground with a grinder attachment of an electric mixer, using the fine adjustment. When the roasts were cubed, moisture was absorbed by the board; the mean loss of moisture was 20 grams. Most of the roasts contained gristle which would not go through the grinder and had to be discarded. The mean weight of gristle was 107 grams, with a range of eight to 339 grams. After grinding twice, the meat was thoroughly mixed by stirring and samples weighed out for analyses. All determinations were made in triplicate.

Freezing of the Meat: The excess raw ground meat was packed in pint containers with cellophane linings which were sealed by heat. The cartons of meat were placed in a freezing unit where the temperature ranged from —15 to —20.6°C.(5 to —5°F.). The meat was stored at this temperature for four or eight months. At the end of these periods the meat was thawed in a refrigerator for approximately 16 hours at a temperature of 5 to 8°C.(41 to 46.4°F.). Chemical determinations were made on the thawed meat and on this meat after pan and oven broiling.

Cooking Frozen Meat: Four patties, each weighing 80 grams, were shaped in a cutter with a diameter of three and one-eighth inches. Preliminary experiments established the following technique whereby the patties would be cooked to the same degree of "doneness" by both oven and pan broiling. Two patties were broiled in a cast-iron skillet preheated at high heat for seven minutes. The heat was then turned down and the patties put in and cooked for two and one-half minutes on each side. The remaining two patties were cooked on a broiler which had been preheated 12 minutes. These patties were two inches from the element and were broiled for two and one-half minutes on each side. There were not sufficient drippings for analysis as the result of either method of broiling.

Preparation of Meat and Drippings for Thiamine and Riboflavin Determinations: Fifty grams of meat were ground with 0.1 N sulfuric acid in a Waring Blendor. The mixture was quantitatively transferred to a 500-milliliter volumetric flask and diluted to volume with 0.1 N sulfuric acid. Aliquots were then taken for analysis.

The drippings were quantitatively transferred to a Waring Blendor and mixed with 0.1 N sulfuric acid. The drippings resulting from pres-

sure saucepan cookery were diluted to 1,000 milliliters, while oven roast drippings were diluted to 500 milliliters.

Thiamine Determinations: A modification of the Hennessy and Cerecedo procedure of Merck and Company, Inc. Laboratories was used for determining thiamine hydrochloride by the direct thiochrome method. Since a Klett fluorimeter with photocell filters 038 and lamp filters 597 was employed, it was necessary to double the amounts of reagents used to prepare aliquots for reading. These were read against a quinine sulfate standard equivalent to one microgram of thiamine hydrochloride per milliliter. Blanks were run on the reagents and sample readings corrected. The amount of thiamine hydrochloride was calculated in terms of the increment in fluorescence resulting from the addition of a known amount of standard thiamine hydrochloride solution to an aliquot of the unknown before oxidation to thiochrome.

Riboflavin Determinations: Riboflavin was determined by the method of Peterson, Brady, and Shaw (1943). However, larger samples of meat than these workers used were weighed out and later dilutions made to adjust the concentration of riboflavin. Solutions to be read were prepared according to Van Duyne, Chase, Owen, and Fanska (1948) with the exception that 0.016 microgram of riboflavin per milliliter was added to Aliquot B. A Klett fluorimeter with photocell filters 351 and lamp filters 5113 was used to determine fluorescence. Difficulty was encountered in the determination of riboflavin in the drippings due to the high percentage of fat in meat of this quality. Consistent values were obtained only after preliminary removal of the fat. These results were too few in number to warrant definite conclusions.

Moisture, Ether Extract, and Protein Determinations: The air oven gravimetric method given by Winton and Winton (1945) was employed to determine the moisture content. Ether extract was determined by the official direct method of the Association of Official Agricultural Chemists (1945). The Kjeldahl-Gunning and Arnold method as given by the Association of Official Agricultural Chemists (1945), and as modified in this laboratory, was used for nitrogen determinations.

RESULTS AND DISCUSSION

Unless otherwise stated the values given are means and standard deviations of the means. The mean weight of the roasts from the right side was 2,348 ± 45 grams compared to 2,371 ± 41 grams of those from the left side. The mean length and width of the 24 roasts were 12.5 ± .17 and 5.3 ± .10 inches, respectively. A summary of the subjective ratings of the 24 raw roasts confirmed the fact they were of prime and choice quality. There was no bone or excess fat on any of the roasts.

Mean cooking time required for the six oven roasts to reach an internal temperature of 82°C.(179.6°F.) was 176 minutes with a range of 168 to 198 minutes. The six roasts in the pressure saucepan were cooked in 54 to 66 minutes with a mean cooking time of 60 minutes. In this case the rise in temperature was gradual and constant, while a mean of 48 minutes was required for the temperature of the oven roasts to climb the last 10 degrees.

Values for the cooking losses are given (Table 1). It is interesting to note that the mean total loss of roasts cooked in the pressure saucepan was significantly [2] higher than that of meat roasted in the oven. These losses are similar to those reported in the literature by Cover (1943) and by Tucker *et al.* (1946).

TABLE 1

Cooking Losses Due to Oven Roasting, Pressure Saucepan Cooking, and Broiling

Meat and method of cooking	No. of cases	Cooking losses	
		Mean and standard deviation of the mean	Range in per cent
		pct.	*pct.*
Top round beef roasts			
Oven roasting			
Loss by evaporation	6	20.3 ± 1.17	17.4-25.4
Loss in drip	6	9.2 ± 1.00	5.3-12.1
Total loss	6	29.5 ± 0.71	27.0-31.7
Pressure saucepan cooking			
Loss by evaporation	6	4.6 ± 1.10	0.1-8.4
Loss in drip	6	31.0 ± 1.90	23.0-37.1
Total loss	6	35.6 ± 1.82	27.7-40.9
Thawed frozen ground beef, top round			
After four months of freezer storage			
Total loss in oven broiling	12	24.1 ± 1.00	17.5-30.6
Total loss in pan broiling	12	17.0 ± 0.62	14.2-21.9
After eight months of freezer storage			
Total loss in oven broiling	12	23.1 ± 2.10	13.8-36.8
Total loss in pan broiling	12	20.2 ± 1.36	12.5-28.7

Pan broiling of the thawed frozen ground meat resulted in smaller cooking losses than did oven broiling. The difference in mean values is significant for the meat broiled after four months of freezer storage, but not for the meat broiled after eight months of freezer storage.

Results of the mechanical tests for tenderness show that the cooked semimembranous muscles with mean readings of 14.3 ± 0.74 and 13.0 ± 0.83 pounds were significantly more tender than the abductor muscles, with mean readings of 17.6 ± 0.69 and 18.8 ± 0.99 pounds. However, there was no significant difference between the mean amounts of force required to shear either muscle according to the method of cooking. These figures correlate well with the scoring of the judges, who gave both types of cooked roasts a mean rating of four for tenderness.

Scores obtained by the subjective judging indicate that the oven roasts were considered more palatable than those cooked in the pressure saucepan. In the former case a mean rating of 37.5 ± 0.92 was obtained when the

[2] Standard deviation of the mean $= S\bar{x}\sqrt{\frac{\Sigma x^2}{n(n-1)}}$

Standard error of the difference $= \sqrt{(S\bar{x}_1)^2 + (S\bar{x}_2)^2}$

A difference is considered significant if it is three or more times as large as the standard error of that difference.

TABLE 2

Thiamine and Riboflavin Content of Fresh and Thawed Frozen Beef Top Round, Before and After Cooking

Meat and treatment	No. of cases	Thiamine		Riboflavin	
		Mean and standard deviation of the mean as determined	Mean total retention[1] and standard deviation of the mean	Mean and standard deviation of the mean as determined	Mean total retention[1] and standard deviation of the mean
		μg./gm.	*pct.*	*μg./gm.*	*pct.*
Fresh top round beef roasts					
Raw roasts paired to those cooked in the oven	6	1.14 ± 0.048	.....	1.92 ± 0.075	.....
Roasts cooked in the oven	6	0.52 ± 0.015	32 ± 2.4	1.54 ± 0.058	56 ± 1.4
Drippings of roasts cooked in the oven	6	0.31 ± 0.044	2 ± 0.3	.....	.....
Total retention after oven roasting	6	.....	34	.....	.....
Raw roasts paired to those cooked in the pressure saucepan	6	1.18 ± 0.090	.....	1.88 ± 0.028	.....
Roasts cooked in the pressure saucepan	6	0.72 ± 0.083	39 ± 3.0	1.43 ± 0.045	48 ± 1.9
Drippings of roasts cooked in the pressure saucepan	6	0.15 ± 0.021	4 ± 0.7	.....	.....
Total retention after pressure saucepan cooking	6	.....	43	.....	.....
Raw fresh top round beef roasts	12	1.16 ± 0.049	.....	1.90 ± 0.039	.....
Thawed frozen ground top beef round					
After four months of freezer storage	12	1.14 ± 0.028	.....	1.94 ± 0.020	.....
After oven broiling	12	1.34 ± 0.029	90 ± 3.4	2.72 ± 0.075	106 ± 2.1
After pan broiling	12	1.25 ± 0.030	92 ± 3.3	2.49 ± 0.028	106 ± 0.8
Thawed frozen ground top beef round					
After eight months of freezer storage	12	1.08 ± 0.040	.....	2.10 ± 0.102	.....
After oven broiling	12	1.21 ± 0.065	86 ± 3.4	2.76 ± 0.145	104 ± 2.7
After pan broiling	12	1.17 ± 0.075	86 ± 3.3	2.60 ± 0.083	102 ± 2.4

[1] Calculated according to $\frac{\text{μg./gm. of cooked meat} \times \text{wt. of cooked meat (or drippings)}}{\text{μg./gm. of raw sample paired to one that was cooked} \times \text{wt. of above meat before cooking}} \times 100$

scores for aroma, texture, tenderness, and flavor of the lean and fat were added and averaged. This value is significantly higher than the rating of 33.6 ± 0.73 given the roasts cooked in the pressure saucepan. The aroma, texture, and tenderness of the meat and the quality of the juice received identical scores in the 12 cooked roasts. There was no significant difference in the color of the lean and fat in the roasts cooked by either method. However, the oven roasts were rated above the roasts cooked in the pressure saucepan on flavor of lean and fat. Without exception, each pressure saucepan cooked roast was rated as drier and less palatable than the corresponding one roasted in the oven. This may well have been caused by the higher cooking losses obtained in the pressure saucepan.

Mean values for the amounts of thiamine and riboflavin present and the percentage retentions of these nutrients are given (Table 2). These retentions were calculated using initial and cooked weights and represent the entire amounts retained. The 12 raw roasts contained 1.16 ± 0.049 micrograms of thiamine per gram. This figure compares well with the amounts reported by Tucker *et al.* (1946) and Booher and Hartzler (1939).

After oven roasting and pressure saucepan cooking, the roasts contained 0.52 ± 0.015 and 0.72 ± 0.083 micrograms of thiamine per gram, respectively. These amounts represent approximately 48 and 61 per cent of the original thiamine on a moist per gram basis. Although the differences between these values are not statistically significant the possibility of greater thiamine retention after pressure saucepan cooking than after over roasting is suggested. The total retentions of thiamine in oven roasted and pressure saucepan cooked meat were 32 ± 2.4 and 39 ± 3.0 per cent, respectively. It is interesting to note that these retentions, calculated using initial and cooked weights, indicate more destruction of thiamine than is usually associated with cooking of roasts. The total retentions of thiamine in the drippings were small, 2 ± 0.3 and 4 ± 0.7 per cent, after oven roasting and pressure saucepan cooking, respectively.

The thawed frozen ground meat did not vary significantly in thiamine content after four and eight months of freezer storage. Broiling of this meat resulted in losses in the amounts of thiamine present, from approximately eight to 14 per cent with no significant difference between thiamine retentions resulting from oven and pan broiling after either period of storage.

The 12 raw roasts had a mean value of 1.90 ± 0.039 micrograms of riboflavin per gram. This amount is similar to that found by Tucker *et al.* (1946). After oven roasting and pressure saucepan cooking, the meat contained 1.54 ± 0.058 and 1.43 ± 0.045 micrograms of riboflavin per gram, respectively. The total percentage retention in oven roasted meat, 56 ± 1.4, is significantly higher than that of pressure saucepan cooked meat, 48 ± 1.9. The mean amount of riboflavin in ground beef did not vary significantly after four and eight months of freezer storage, nor was there any loss of riboflavin during pan and oven broiling. The apparent gains in the amounts present fall within the experimental error of the method.

Mean values for the moisture, ether extract, and protein contents are given (Table 3). Percentages of moisture, ether extract, and protein in

TABLE 3

Moisture, Protein, and Ether Extract Content of Fresh and Thawed Frozen Beef Top Round, Before and After Cooking[1]

Meat and treatment	No. of cases	Moisture		Protein		Ether extract	
		Mean and standard deviation of the mean	Range in per cent	Mean and standard deviation of the mean	Range in per cent	Mean and standard deviation of the mean	Range in per cent
		pct.	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*
Fresh beef top round							
Roasts paired to those cooked in the oven	6	67.1 ± 0.68	65.3-70.9	19.6 ± 0.24	19.0-20.2	12.6 ± 1.02	11.3-15.7
Roasts paired to those cooked in the pressure saucepan	6	66.5 ± 1.44	62.5-68.0	19.4 ± 0.40	17.6-20.4	15.0 ± 1.72	13.2-18.5
Mean of raw roasts	12	66.8 ± 0.76	62.5-70.9	19.5 ± 0.23	17.6-20.4	13.7 ± 0.98	11.3-18.5
Roasts after oven roasting	6	54.1 ± 0.87	51.7-56.3	28.4 ± 0.48	26.8-29.6	14.3 ± 1.42	11.1-19.0
Roasts after pressure saucepan cooking	6	52.5 ± 0.98	49.3-54.6	29.0 ± 0.36	28.2-30.0	15.6 ± 1.81	10.3-20.7
Thawed frozen ground beef top round							
After 4 months of freezer storage							
Raw	12	67.2 ± 0.55	64.1-70.5	19.6 ± 0.18	18.7-20.7	11.9 ± 0.40	10.6-14.6
After oven broiling	12	58.4 ± 0.74	51.7-60.9	25.4 ± 0.44	22.4-26.9	13.9 ± 0.56	12.4-17.3
After pan broiling	12	60.2 ± 0.81	54.9-62.6	23.8 ± 0.25	22.3-25.2	13.5 ± 0.62	9.6-16.6
After 8 months of freezer storage							
Raw	12	65.7 ± 0.39	64.2-68.3	19.5 ± 0.14	19.0-20.5	14.4 ± 0.76[2]	12.0-15.4
After oven broiling	12	57.7 ± 0.86	53.2-62.6	24.5 ± 0.61	20.9-26.5	15.4 ± 1.15[2]	11.9-18.4
After pan broiling	12	59.1 ± 0.74	53.8-62.8	24.2 ± 0.48	21.6-27.1	16.0 ± 1.41[2]	12.0-18.3

[1] These per cents are on the "as determined" basis. [2] Mean of five cases.

the row top rounds of beef are 66.8 ± 0.76, 18.7 ± 0.98, and 19.5 ± 0.23, respectively. These figures are similar to those given by Chatfield and Adams (1940) for top round of beef. Cooking and freezer storage affected the moisture, ether extract, and protein contents, as might be expected.

SUMMARY

Twelve comparable top round beef roasts were cooked in the pressure saucepan or roasted in the oven to an internal temperature of 82°C. (179.6°F.). The amounts of thiamine, riboflavin, moisture, ether extract, and protein in the cooked roasts and in the paired roasts which were analyzed raw were determined. Mean values obtained for cooking times, cooking losses, tenderness, palatability, and vitamin retention support the following conclusions:

Top round beef roasts, weighing approximately five pounds each, were cooked in the pressure saucepan in approximately one-third of the time required for oven roasting.

While the greater proportion of the weight loss of the meat cooked in the oven was due to evaporation, roasts cooked in the pressure saucepan had significantly greater losses in drip and total cooking losses than those roasted in the oven.

No difference could be detected by a mechanical shear stress apparatus or by subjective judging in the tenderness of semimembranous and abductor muscles of beef top round cooked by either method.

When top round beef roasts were cooked in the oven, more palatable meat was obtained than when similar roasts were cooked in the pressure saucepan. The difference between the mean subjective ratings was small but significant. The judges preferred the flavor of the lean and fat of the meat roasted in the oven and considered the meat cooked in the pressure saucepan too dry.

Roasts cooked in the pressure saucepan retained more thiamine and less riboflavin than similar cuts of meat roasted in the oven. On the basis of total percentage retentions the former difference is not significant while the latter is. The total amounts of thiamine in the drippings after either method of cooking represent small proportions of the amounts originally present in the raw roasts.

Analyses of the thawed frozen ground beef top round showed that thiamine and riboflavin contents were not significantly changed during freezing, four or eight months of freezer storage, and thawing. Retentions of thiamine and riboflavin during oven or pan broiling were approximately 88 and 104 per cent, respectively.

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CRACKED ICE AND PRESERVATION OF STORED FRUITS AND VEGETABLES[1,2]

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This investigation was undertaken in order to gain further information concerning the changes in quality of foodstuffs between the time of harvesting and the time they are processed for consumption or for storage. Growers in some states make extensive use of cracked ice in direct contact with produce shipped in trucks and refrigerator cars. Commodities grown for local or nearby consumption, however, seldom receive protection against losses in quality, although frequently many hours elapse between time of harvest and consumption. Since crushed ice is now commercially available, it seemed desirable to determine the extent to which it will preserve the quality of fruits and vegetables through various stages of handling and storage.

Two criteria were used in this study: consumer ratings (appearance, flavor and aroma, texture, and general palatability) and retention of ascorbic acid. It was suggested by Fenton (1940) that loss of ascorbic acid from fruits and vegetables is an excellent index of deterioration in their nutritive value. Since this vitamin is both labile and soluble, one would expect its retention in stored or processed food to parallel the retention of other vitamins which might also be lost easily. For this reason, and because fruits and vegetables are the important sources of ascorbic acid in the diet, assays were made to trace the losses of this vitamin from several foodstuffs. Since recent research indicates that both reduced ascorbic acid and dehydro-ascorbic acid are effective as a source of vitamin C, it was deemed desirable to make quantitative estimations of both forms.

Three foods were chosen for the test: (1) Strawberries, a favorite but easily perishable fruit; (2) peas, a legume product, the pods of which are usually discarded before cooking; and (3) snap beans, another legume product, the pods (modified leaves) of which are usually not discarded.

Mayfield and Richardson (1943) found that strawberries (Dunlap and Gem varieties) contained 70 mg. reduced ascorbic acid per 100 grams, and did not examine the fruit for the dehydro-form. There was no appre-

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ciable loss of the vitamin when fresh strawberries were held for five days at 4.4°C.(40°F.) or for two days at room temperature, 23.9°C.(75°F.).

Similar observations on vegetables have been published by several laboratories. Pyke and Allison (1946) studied the changes in content of ascorbic acid, sugar, and starch in peas stored with "snow-ice" or at room temperature. The iced peas lost only 15 per cent of their reduced ascorbic acid in three days; peas stored in air at room temperature, 21.1 to 23.9°C.(70 to 75°F.), during the same period lost 65 per cent of their reduced ascorbic acid. Peas gained in total sugars and starch when stored three days with "snow-ice." The authors assumed that food material was transferred from the pods to the edible portions. Peas stored in air at room temperature lost sugar and gained starch. Patton and Miller (1947) found that peas which contained 39 mg. of reduced ascorbic acid per 100 grams when harvested, retained more than 95 per cent of the vitamin when stored with ice for five days. Peas stored at room temperature, 27.4°C.(80°F.), lost more than one-half their reduced ascorbic acid content during the same period.

Mack, Tapley, and King (1939) reported a loss of 46 per cent of the ascorbic acid from green beans after 24 hours at room temperature, 21 to 23°C.(69.8 to 73.4°F.). Storage at room temperature for six days resulted in losses of 58 to 81 per cent of the vitamin in four varieties of snap beans. They found that beans stored at 1 to 3°C.(34 to 37.4°F.) lost 42 to 65 per cent of their ascorbic acid on six days' storage. Gordon, Griswold, and Porter (1945) found that snap beans lost only 2.6 per cent of their reduced ascorbic acid when stored for eight hours in "snow-ice." The beans lost 11 per cent of the vitamin during storage at room temperature, 21.1 to 30°C.(70 to 86°F.), for the same period. Tests conducted by Bryson (1945) revealed that freshly harvested snap beans showed no loss in reduced ascorbic acid after from two to three days' storage in contact with ice. Storage for two days at room temperature resulted in loss of 35 per cent of the vitamin; after three days' storage at room temperature 42 per cent of the ascorbic acid had disappeared.

MATERIALS AND METHODS

Commodities Assayed: All fruits and vegetables used in this investigation came from the University gardens. The strawberries (Blakemore variety) were harvested late in May. The fruit, packed in commercial boxes and crates, was held in a mechanical refrigerator at 4°C.(39.2°F.) for 41 hours before the beginning of the experimental storage period. The peas (Thomas Laxton variety) were also harvested late in May. They were brought to the laboratory immediately after harvesting and packed at once for storage. Tests were made twice with snap beans (Landreth Stringless Green Pod): the beans tested in September were brought to the laboratory immediately after harvesting, but those used in June were held in a mechanical refrigerator at 4°C. for 21 hours before the beginning of the experimental storage period.

Storage and Sampling: The boxed berries were divided into two similar lots. Half of the berries was kept above crushed ice, in a closed, insulated refrigerator, for five days; the other half was stored at room temperature

until none of the berries was edible. Boxes of fruit were removed for sampling at 24-hour intervals and washed and dried with towels. Records were kept of the weight of edible fruit.

The peas were divided into 750-gram lots, taking care to make these portions as uniform as possible in maturity and size of pods. Each 750-gram portion was wrapped loosely in cheesecloth to keep it a unit throughout the experiment. Half of the wrapped portions was packed loosely in a bushel-basket for storage at room temperature; the other wrapped portions were placed on the wire tray of a refrigerator. The floor was covered by a deep layer of cracked ice. At the beginning of the experiment every cheesecloth-enclosed portion was surrounded by cracked ice. On each succeeding day fresh cracked ice was placed on top of the stored, iced vegetables to replace the ice and cold water that dripped away. Storage and sampling of the snap beans were identical with those for the peas except that the beans were stored in 500-gram portions.

TABLE 1

Fluctuations in Outdoor Temperature During Storage Periods

Date	Temperature range	Date	Temperature range	Date	Temperature range
	°F.		°F.		°F.
9- 4-'45	97-71	5-25-'46	63-54	6-27-'46	93-70
9- 5-'45	97-68	5-26-'46	69-51	6-28-'46	90-72
9- 6-'45	91-64	5-27-'46	77-47	6-29-'46	87-72
9- 7-'45	85-72	5-28-'46	81-52	6-30-'46	84-70
9- 8-'45	93-71	5-29-'46	76-58	7- 1-'46	84-65
9- 9-'45	96-68	5-30-'46	80-60	7- 2-'46	83-60
9-10-'45	79-57	5-31-'46	79-62	7- 3-'46	86-60
.....	.....	6- 1-'46	75-50	7- 4-'46	90-59
.....	.....	.....	.....	7- 5-'46	93-65

The vegetables were sampled daily and records made of the weights of the unshelled pods of peas and beans and of edible shelled peas. The vegetables were cooked and judged for quality (consumer rating scores) by members of the University Department of Home Economics. Chemical determinations were made only upon raw fruits and vegetables. Materials for vitamin assays were sharp-frozen as they were removed from storage daily and were held at —40° (F.,C.) until analyzed.

Unfortunately, the room temperatures registered by the automatic temperature recorder turned out to be erroneous. Data supplied by the Weather Bureau, giving maximum and minimum outdoor temperatures for each day, are summarized (Table 1). Probably the highest temperature in the storage room during each day was several degrees below the maximum outside temperature, and the lowest temperature in the room during the night was at least several degrees above the minimum temperature of the outdoor air.

Chemical Methods: Ascorbic acid determinations on strawberries were made by the xylene extraction method of Robinson and Stotz (1945). The Mack and Tressler (1937) modification of Tillmans' method was used in the determination of ascorbic acid in the vegetables. Assays for total

ascorbic acid were made upon extracts which had been saturated with H_2S, stored overnight in the refrigerator, and freed from H_2S by bubbling N_2 gas through the solutions.

Samples for vitamin assay were taken from frozen composites made up of 175 grams or more of strawberries, 160 grams or more of shelled peas, and 200 grams or more of snap beans. (Because of spoilage the composite from the last sample of beans stored at room temperature contained only 120 grams of the vegetable.) The frozen material for each composite was ground with a food chopper, mixed quickly but thoroughly, then returned to storage at —40° (F., C.) until assayed. When the assay was begun the ground material was weighed in the frozen state and kept frozen until placed in the extractant.

Consumer Rating: Daily cooking tests were made on samples of raw shelled peas, 100 grams in weight, which had been held at room temperature or in chipped ice. Each day a sample of "garden-fresh" vegetable was cooked for comparison with the stored samples. A pressure saucepan was used in cooking. Water (110 grams, approximately one-half cup) and three grams of salt were added to the vegetable before cooking. Gas heat was used. The maximum temperature was reached in five minutes and the peas were cooked one minute at 15 pounds' pressure (250°F.). The pan was immediately placed in cold water to stop the cooking process and the peas removed and drained.

Similar cooking tests were made daily upon raw snap beans stored at room temperature or in chipped ice. The samples cooked on each of the first three days weighed 200 grams. With subsequent deterioration of the commodity it was necessary to reduce to 150 grams the amount of raw snap beans in each cookery test during the last three days. The beans were cooked two minutes at 15 pounds' pressure (250°F.) after reaching the maximum temperature in five minutes.

Three or four trained judges scored each of the cooked samples while it was still warm. Samples were scored upon the basis of odor, color, appearance, texture, flavor, and general condition. The subjective ratings of the judges were changed to numerical scores on the basis of the following evaluation: Very desirable—5, desirable—4, slightly desirable—3, slightly undesirable—2, undesirable—1. Averages of the scores of the judges are summarized (see tables of results).

Representative samples of raw and cooked vegetables were, for comparison, checked for color with charts in a "Dictionary of Color" by Maerz and Paul (1930).

RESULTS AND DISCUSSION

Strawberries: As would be expected, berries held over ice remained edible for a longer time than those stored at room temperature. The iced fruit remained bright and plump while the other fruit quickly became dull and shrunken, with wilted sepals. Mold growths appeared more quickly and were more extensive on berries held at room temperature. This difference in rate of deterioration is shown clearly in the percentage of fruit considered unfit for consumption at each sampling (recorded Table 2).

The tests show that strawberries are an excellent source of ascorbic acid, though the amount varies from box to box, and that it is present in both dehydro- and reduced forms. The results of the assays also indicate that there is, within experimental error, no appreciable change in the content of reduced ascorbic acid or of total ascorbic acid in the strawberries while they remain in an edible condition. This is true whether the berries are iced or held at room temperature.

TABLE 2

Ascorbic Acid in Strawberries (Blakemore Variety)[1]

Storage time[2]	Unfit for consumption	Ascorbic acid (calculated on basis of fresh weight)		
		Reduced	Dehydro-	Total
Stored over ice				
hr.	*pct.*	*mg./ 100 gm.*	*mg./ 100 gm.*	*mg./ 100 gm.*
0	00.00	41.7	16.6	58.3
24	15.50	54.9	6.2	61.1
48	24.20	.....	.....	.....
72	44.20	46.1	24.3	70.4
96	58.90	54.9	1.1	56.0
120	51.50	52.4	17.2	69.6
Stored at room temperature				
24	45.60	51.2	12.3	63.5
48	60.50	47.7	20.0	67.7
72	96.75	.....	.....	.....

[1] Ripened during cloudy, rainy weather. [2] Stored from 5-25-46 to 5-30-46.

Peas: The iced peas were markedly more attractive in appearance than those stored at room temperature. The pods of these peas had an excellent color and remained bright, crisp, plump, turgid, and easily shelled until the end of the experiment. The pods of the peas stored at room temperature soon became dull, flaccid, and flat, and by the fifth day had a yellow cast. The iced peas showed a slight gain in weight with storage, while the other peas continuously lost weight. Results of the tests with peas are summarized (Tables 3 and 4). Chemical assays of the shelled peas indicate the presence of ascorbic acid in both reduced and dehydro-forms. With storage the dehydro-compound disappears more quickly than the reduced acid. The iced peas show excellent retention of total ascorbic acid. The content of the vitamin in the shelled peas remains constant during the first three days; at the end of the experiment 90 per cent or more of the vitamin (calculated on a dry weight basis) has been retained by the iced peas.

Storage at room temperature results in a marked decrease in total ascorbic acid. By the end of 24 hours a third of the vitamin is lost and by the fourth day over half is destroyed.

The peas held at room temperature had developed a starchy taste at the end of the second day. By the end of the fourth day this was more pronounced and they had lost their characteristic taste and odor. By the fifth day, the judges considered them undesirable but not inedible.

TABLE 3

Changes in Peas During Storage (Thomas Laxton Variety)

Storage time[1]	Original weight	Weight after storage		Dry matter in shelled peas	Retention of total ascorbic acid (dry weight basis)	General quality rating[2]
		Whole unshelled pods	Shelled peas			
Stored with ice						
hr.	*gm.*	*gm.*	*gm.*	*pct.*	*pct.*	
0	750	.....	.....	19.9	100.0	.....
24	750	793.5	278.2	20.2	100.0	3.67
40	750	780.0	266.2	18.6	100.0	3.67
64	750	799.0	279.4	19.0	100.0	2.67
88	750	811.1	295.6	19.8	90.1	3.00
112	750	801.7	307.0	19.9	93.3	2.67
Stored at room temperature						
24	750	720.0	262.5	20.4	66.5	3.67
40	750	707.3	269.3	21.0	61.9	3.67
64	750	666.9	280.1	20.0	69.5	2.67
88	750	648.8	271.1	22.0	48.4	2.67
112	750	613.7	296.3	23.8	46.7	1.33

[1] Stored 5-27-46 to 6-1-46. [2] Very desirable—5, desirable—4, slightly desirable—3, slightly undesirable—2, undesirable—1.

TABLE 4

Vitamin Retention and Consumer Ratings of Stored Peas (Thomas Laxton Variety)

Storage time[1]	Ascorbic acid (calculated on basis of fresh weight)			Consumer rating scores[2]				
	Reduced	Dehydro-	Total	Odor	Color	Appearance	Texture	Flavor
Stored with ice								
hr.	*mg./100 gm.*	*mg./100 gm.*	*mg./100 gm.*					
0	26.0	10.6	36.6	.....	.....	.....	.....	.....
24	26.1	12.0	38.1	3.00	4.67	3.00	3.00	3.67
40	26.6	17.0	43.6	3.00	4.33	3.67	3.33	4.00
64	27.4	8.3	35.7	3.67	4.33	4.33	2.33	2.67
88	24.6	8.1	32.7	2.33	3.33	3.00	2.67	3.33
112	22.3	11.7	34.0	2.33	2.67	2.67	2.33	3.33
Stored at room temperature								
24	16.4	8.5	24.9	2.33	4.67	3.33	3.67	3.67
40	16.8	7.0	23.8	2.67	3.67	4.00	3.33	3.67
64	19.0	6.5	25.5	2.67	4.33	4.33	2.33	2.67
88	15.9	3.6	19.5	2.67	2.67	3.67	2.67	2.33
112	16.2	4.1	20.3	1.67	2.33	2.67	1.33	1.33

[1] Stored 5-27-46 to 6-1-46. [2] Very desirable—5, desirable—4, slightly desirable—3, slightly undesirable—2, undesirable—1.

They held their shape and had no slipped skins, but were faded and not uniform in color.

The peas held in chipped ice were still considered acceptable at the end of the fifth day. The odor was less pronounced, the texture slightly mushy, and the flavor not as full or characteristic as that of the fresh product.

Snap Beans: The better appearance and quality of the iced beans were clearly apparent from the beginning of the investigation. At the end of the test the pods of these beans were still crisp and green; the seeds remained green, without the characteristic pigmentation of ripening seeds. By the end of three days some of the beans held at room temperature had become slimy and some pods had developed molds. The pods that did not grow moldy became more dry and limp, and with increasing storage grew more tough and rubbery. A yellow color was evident in the pods. By the fifth day the seeds showed the blue pigments associated with maturity.

Information on ascorbic acid content was obtained for two series of stored beans (Table 5 shows vitamin data for beans stored in June; Table 6, similar data from tests in September. As indicated by the dry matter in the original samples, the June beans were less mature than the beans stored in September.)

Data (see Table 5) show the decrease in weight of snap beans (unshelled pods) considered edible after storage at room temperature. Toward the end of the experiment the pods from these beans were dry and inedible and to salvage any usable portion the seeds would need to be shelled out. On the other hand, the weight of edible material from the iced beans remained constant throughout the course of the experiment.

Our data indicate the presence of ascorbic acid in both reduced and dehydro-forms in the stored snap beans. Retention of ascorbic acid (total and reduced) is much better in iced beans than in those held at room temperature, if the content of the vitamin is calculated on a dry weight basis (see Tables 5 and 6).

In contrasting results of the tests with snap beans and with peas it is to be remembered that in testing the beans, whole, unshelled pods were assayed; in assaying the peas, shelled seeds, only, were checked. As a rule the amount of ascorbic acid in legumes, as shown by Flynn, Hibbard, Hogan, and Murneek (1946), is higher in the seeds than in the shelled pods. When stored at room temperature the seeds of both beans and peas matured, which resulted in an increase in their content of ascorbic acid. Such an increase would tend to counteract the destruction of ascorbic acid in tissues stored at room temperature.

Beans held at room temperature for two days were adjudged undesirable when cooked, but those held in chipped ice were still considered edible at the end of six days. They had, however, developed an unnatural sweet flavor. To some of the judges this sweet flavor was not objectionable, while others considered it very undesirable. Subsequent chemical tests on green beans showed that sugars developed in beans during storage with ice. As recently reported by Williams, Flynn, and Hogan (1948), sucrose made up the larger portion of these sugars.

TABLE 5

Changes in Snap Beans During Storage
(Stringless Green Pod Variety)

Storage time[1]	Stored beans		Ascorbic acid (calculated on basis of fresh weight)			Retention of total ascorbic acid (dry weight basis)
	Weight of usable beans	Dry matter	Reduced	Dehydro-	Total	
			Stored with ice			
hr.	*gm.*	*pct.*	*mg./100 gm.*	*mg./100 gm.*	*mg./100 gm.*	*pct.*
0	500.0	10.5	21.9	13.3	35.2	100.0
18	508.0	9.9	19.4	10.6	30.0	90.6
42	505.5	10.2	18.7	5.7	24.4	71.6
90	523.0	9.8	17.5	0.7	18.2	55.1
114	525.0	8.6	15.7	1.8	13.9	48.5
136	527.9	9.1	14.0	1.0	15.0	49.1
184	532.0	8.9	10.3	2.2	12.5	41.8
			Stored at room temperature			
18	440.0	11.1	18.9	2.5	21.4	57.6
42	430.0	11.0	16.0	10.5	26.5	71.9
90	404.1	11.6	11.7	5.6	17.3	44.7
114	382.0	11.2	12.3	6.2	18.5	49.5
136	383.3	11.8	10.3	3.6	13.9	35.2
184	272.0	15.7	8.6	10.2	18.8	35.8

[1] Stored 6-27-46 to 7-5-46.

TABLE 6

Vitamin Retention and Consumer Ratings of Stored Snap Beans
(Stringless Green Pod Variety)

Storage time[1]	Results of chemical determinations			Consumer rating scores[2]					General conclusions
	Dry matter	Reduced ascorbic acid (fresh weight basis)	Retention of reduced ascorbic acid (dry weight basis)	Odor	Color	Appearance	Texture	Flavor	
				Stored with ice					
hr.	*pct.*	*mg./100 gm.*	*pct.*						
0	13.1	27.3	100.0	5.0	5.0	5.0	5.0	5.0	5.0
8	12.4	29.7	100.0	.....	.....	.....	.....	.....	.....
24	12.5	29.5	100.0	4.0	4.0	5.0	3.6	4.0	4.0
48	12.8	26.5	90.4	4.0	5.0	5.0	4.0	4.0	4.0
72	11.9	24.0	87.8	4.0	4.0	4.0	4.0	3.5	4.0
96	11.7	21.4	76.3	4.0	4.5	4.5	4.5	3.5	4.0
120	12.6	22.2	77.3	3.0	4.0	4.5	3.5	3.0	3.0
144	11.1	20.4	84.5	3.0	4.0	4.5	4.0	2.5	3.0
				Stored at room temperature					
8	12.8	31.1	100.0	.....	.....	.....	.....	.....	.....
24	13.5	21.7	69.9	4.0	4.0	4.0	4.0	3.0	4.0
48	13.1	20.0	66.3	2.4	3.5	4.0	3.0	2.0	3.0
72	14.9	17.5	51.1	2.4	3.0	3.5	2.0	1.5	3.0
96	14.6	14.7	44.1	2.4	2.5	3.0	2.0	2.0	2.5
120	13.5	11.3	36.6	1.0	1.0	1.0	1.0	1.0	1.0
144	15.5	8.9	25.2	1.0	1.0	1.0	1.0	1.0	1.0

[1] Stored 9-4-45 to 9-10-45. [2] Very desirable—5, desirable—4, slightly desirable—3, slightly undesirable—2, undesirable—1.

Beans held at room temperature showed little change in color during the first three days, but after that time the pods began to turn yellow-green and by the sixth day approximately 50 per cent was definitely yellow. Raw beans held in chipped ice showed slight change in color. When they were cooked these beans had an intense green color and remained attractive in appearance until the sixth day, when a yellowish cast became noticeable.

SUMMARY AND CONCLUSIONS

Stored strawberries retain their high ascorbic acid content (58 to 70 mg./100 grams fresh weight) as long as they remain edible, regardless of whether they are chilled or stored at room temperature. However, refrigeration markedly retards deterioration.

Unshelled peas held under cracked ice for five days retained 90 per cent of their original ascorbic acid content (37 mg./100 grams fresh weight). Approximately one-third of the total ascorbic acid in the iced peas was present in the dehydro-form. When stored at room temperature peas lost half of their total ascorbic acid by the end of the fourth day. As the length of the storage time increased there was a gradual loss of dehydro-ascorbic acid from the peas held at room temperature, but the amount of reduced ascorbic acid in these peas remained constant. This may have been the result of ripening of the seeds at room temperature, with the attendant increase in reduced ascorbic acid known to occur in the seeds of legumes during the ripening process.

Snap beans harvested in the early summer (June) contained 22 mg. of reduced ascorbic acid and 35 mg. of total ascorbic acid per 100 grams fresh weight. Beans harvested in the early fall (September) were at a later stage of maturity. Assays showed they contained 27 mg. of reduced ascorbic acid per 100 grams fresh weight (total ascorbic acid was not determined). Making calculations on a dry basis, beans harvested in June retained 55 per cent of their reduced ascorbic acid and 42 per cent of their total ascorbic acid when stored with ice for eight days. Beans stored at room temperature at the same time retained 26 per cent of their reduced ascorbic and 35 per cent of their total ascorbic acid, on a dry basis. When the beans harvested in September were stored in cracked ice for six days they retained 88 per cent of their original content of reduced ascorbic acid. Beans stored at room temperature during the same period retained 28 per cent of their original content of reduced ascorbic acid, on a dry weight basis. It is not easy to explain why the beans stored in ice in September retained a larger fraction of their original content of reduced ascorbic acid than those stored in ice in the early summer. The explanation may lie in the more advanced maturity of the beans harvested in the fall.

The iced vegetables gradually imbibed water during storage and gained slightly in weight, but the vegetables stored at room temperature gradually lost weight. Consumer rating tests (scoring odor, color, appearance, texture, and flavor) confirmed the superior quality of the iced vegetables, as shown by chemical tests.

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AN IMPROVED MICROSCOPIC METHOD OF EXAMINING FATTY FOODS[1]

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Microscopic methods of examination of food products as a supplement to cultural tests have been used for many years with success, in spite of many inherent limitations to the tests. The merit of such a test lies in its ability to reveal the presence of large numbers of organisms killed during the processing of the raw materials into the finished food. As a check on the sanitary condition of a food under such conditions, the microscopic examination may show that raw products of low quality were employed, even when cultural tests indicate the finished product was of high quality. Examination of fatty acids, such as butter and oleomargarine, has been beset with difficulties of getting the sample adequately homogenized to give uniform results in the microscopic test.

During the routine microscopic examination of a large number of samples of oleomargarine, it was found preparations obtained using Fay's method (1935) were unsatisfactory for determining the number of bacteria in this fatty food. Fay's method was originally devised for use in butter examination. Smears of oleomargarine prepared in this manner were found to be lumpy and very uneven in appearance even when great pains were taken to attain homogenization of the sample with the fixative. After staining, a satisfactory microscopic examination of the material was almost impossible. The stained film showed large areas with no apparent material and other areas with clumps of bacteria and curdy residue. Apparently the natural immiscibility of xylol-fat mixtures and aqueous solutions accounts for the difficulty in obtaining smooth smears by Fay's technique.

Snieszko (1942) reported on the use of detergents in the staining of bacteria. His experience indicated the addition of small amounts of Aerosol OT or Aerosol MA 1:500 to a bacterial suspension would make it possible successfully to use mechanically instead of chemically cleaned slides for many bacteriological staining techniques, including the flagella stain.

It was, therefore, reasoned that if the incorporation of a detergent would give better spreading of a bacterial smear on only "roughly" cleaned slides, perhaps the addition of a detergent to the oleomargarine film might aid in the homogenization of the sample, and give a smoother film with more uniform distribution of the contained bacteria.

A surface-active agent, "Tide" (a household detergent manufactured by Proctor & Gamble Company), was found to be effective in the prep-

[1] Contribution No. 246, Department of Bacteriology, Kansas State College, Manhattan, Kansas.

aration of a well-emulsified, homogeneous film which stained evenly and facilitated microscopic examination. The procedure outlined below was found to be satisfactory:

(a) Melt the sample of oleomargarine at 45°C.(113°F.) within a 15-minute period. Shake thoroughly.

(b) Place 0.1 ml. of the sample on a standard glass slide and add one drop of Mayer's solution and three drops of a one per cent "Tide" solution.

(c) Mix vigorously with an "L"-shaped inoculating needle and then smear over the entire area of the slide.

(d) Fix quickly by passing the slide through the flame of the Bunsen burner three times. This is done rapidly so that the oil droplets will not coalesce into large areas but will remain minute and evenly distributed over the slide.

(e) Drip xylol over the slide to defat the smear, then immerse the slide in 95 per cent alcohol from 30 to 40 seconds. Dry in air.

(f) Again drip xylol over the smear and dry in the air. Repeat until the smear is "cleared"; then dry.

(g) Stain with Loeffler's alkaline methylene blue stain for about 30 seconds.

(h) Wash by dipping the slide into two jars of water. Allow to dry at room temperature.

(i) Examine under the oil immersion lens, the field of which has been standardized to a diameter of 0.157 mm. The average number of organisms in 100 fields multiplied by 1,000,000 equals the number of organisms per milliliter of melted sample.

The improved appearance of the finished smear and the greater consistency of results have led the authors to submit this method for general use by others. Although "Tide" is not a pure chemical compound, its general availability and low cost made it seem a desirable ingredient to use, especially under field conditions. Possibly any other surface-active agent or detergent would prove equally useful, provided it did not cause disruption of the cells or alter the staining properties of the smears.

SUMMARY

An improved method for making smears of fatty foods for microscopic examination for bacteria is reported.

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EFFECT OF FREEZING AND FREEZER STORAGE ON THE ASCORBIC ACID CONTENT OF MUSKMELON, GRAPEFRUIT SECTIONS, AND STRAWBERRY PUREE

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This study was undertaken to determine the effect of freezing and freezer storage on the ascorbic acid content of muskmelon, grapefruit sections, and strawberry purée and to investigate certain variables in procedure which might influence the amounts retained. Fresh muskmelon, grapefruit, and strawberries are good sources of ascorbic acid. Muskmelon, often referred to as cantaloupe, was among the fruits selected for study because it is commonly grown in this region, but is not available throughout the year. Grapefruit was included in order to find out if the ascorbic acid content is as stable during freezing and freezer locker storage as under other conditions, and strawberry purée was used to determine the amounts of ascorbic acid retained when it was packed with two concentrations of sugar in two types of containers. There are no reports in the literature which deal with the effect of freezer storage on the retention of ascorbic acid in muskmelon and grapefruit. However, several studies have been carried on to determine the amounts of ascorbic acid in the fresh fruit.

Floyd and Fraps (1939) determined the ascorbic acid content of six varieties of cantaloupe and found average values ranging from 7.3 to 37.1 milligrams per 100 grams present. Six varieties of cantaloupe assayed by Wheeler, Tressler, and King (1939) contained from 0.23 to 0.48 milligram of ascorbic acid per gram of fresh fruit. Moseley and Satterfield (1940) reported a wide variation in the amounts of ascorbic acid present in six varieties purchased on the market, as well as in different samples of the same variety. Average values for the varieties tested ranged from 20.91 to 31.23 milligrams per 100 grams. In a study of 16 varieties of muskmelons grown in Ohio, Wagner, Hoffman, and Brown (1940) found that the average ascorbic acid content varied from 0.16 to 0.57 milligram per gram. Smith, Farrankop, Caldwell, and Wood (1944) tested 141 melons of three crate sizes. The average amount of ascorbic acid present was 42 milligrams per 100 grams and the values ranged from 25 to 63 milligrams, independent of size.

Average values for the ascorbic acid content of six varieties of grapefruit determined by Floyd and Fraps (1939) ranged from 31.3 to 44.1 milligrams per 100 grams. Metcalfe, Rehm, and Winters (1940) also tested six varieties of fresh grapefruit and found similar values ranging from 32.0 to 37.7 milligrams per 100 cubic centimeters of juice. Smith, Ross, and Caldwell (1944) reported that segments and salad sections of Marsh grapefruit contained 31 and 35 milligrams of ascorbic acid per 100 grams, respectively.

Several papers have been published which deal with the effects of freezing, freezer storage, and thawing on the ascorbic acid content of whole and sliced strawberries, prepared for freezing in plain, dry sugar, or sugar sirup packs. Loeffler (1946) reviewed this work and only studies directly pertinent to the present one will be referred to here. Kirk and Tressler (1941) tested frozen, pulped strawberries to which sugar had been added. They found that after one month of freezer storage losses of ascorbic acid were inversely proportional to the amounts of sugar added when these amounts were between 50 and 25 per cent, even when compared on a sugar-free basis. Loeffler (1946) reported that puréeing of strawberries or flash-pasteurization of this purée did not decrease the ascorbic acid content more than five per cent. Purée to which sugar had been added lost 12 per cent during freezing, three months of frozen storage, and defrosting, with an additional 10 per cent loss when the storage period was extended to seven months.

The present work was planned to determine the amounts of ascorbic acid retained in thawed muskmelon, grapefruit sections, and strawberry purée after freezing and different intervals of freezer storage. Bender's Surprise, Hale's Best, and an unknown variety of muskmelon and sections of grapefruit were packed plain or covered with sugar sirup. Strawberry purées were prepared using five and three parts of purée to one part of sugar and were packed in glass freezer jars or cellophane-lined cartons. Reduced ascorbic acid determinations were made on all samples and total ascorbic acid determinations on one series of muskmelon.

EXPERIMENTAL PROCEDURE

In 1945 two varieties of muskmelon, Bender's Surprise and Hale's Best, were grown under the supervision of the Department of Horticulture of the University of Illinois. The melons were harvested at suitable stages of maturity for freezing and were brought to the food research laboratory for processing on the day they were picked.

The muskmelons were halved and the seeds taken out. Half of each melon was used for the sirup-packed fruit and the other for the plain-packed fruit. The halves were sliced, rind removed, and slices cut into "bite-size" wedges. Three hundred and ten-gram lots of fruit were packaged in heat-sealable, cellophane-lined pint containers without the addition of sugar or sugar sirup (plain-packed) or with 150 milliliters of 40 per cent sugar sirup. This procedure was carried out on four days. Determinations were made of the reduced ascorbic acid content of the fresh fruit and of the muskmelon after approximately 24 hours in the freezing unit and thawing, and after freezing followed by one, three, six, and nine months of freezer storage and thawing. The containers of frozen muskmelon were held overnight in a refrigerator so that the fruit would be thawed in time for sampling. Samples consisted of 150 grams of fresh material, 175 grams of plain-packed wedges, or one-third by weight of the solid and liquid portions in a pint container of sirup-packed fruit. These were ground in a Waring Blendor with three per cent metaphosphoric acid, the mixtures were filtered, and aliquots were diluted for titration. Reduced ascorbic acid was determined by the titration method of Till-

mans, Hirsch, and Hirsch (1932) as modified by Bessey and King (1933) and Bessey (1938).

An unknown variety of muskmelon was purchased on the market in 1946. These melons were prepared for freezing according to the procedure used the previous year, except that the amount of sugar sirup per pint container was increased to 175 milliliters. Determinations were made after the same intervals. One hundred-gram samples of fresh fruit, one-third of a container of plain-packed fruit, and one-fifth of the liquid and solid portions of the sirup-packed fruit were weighed out. Reduced ascorbic acid was determined by the Loeffler and Ponting (1942) method after grinding the sample with one per cent metaphosphoric acid in a Waring Blendor. Total ascorbic acid was determined by a combination of the Bessey (1938) method and that of Loeffler and Ponting (1942).

Medium-white grapefruit from Texas was purchased on the local market in March, 1946. The grapefruit was peeled, sectioned, and apportioned into 11 lots; the ascorbic acid content of one of these was determined immediately. Five lots of grapefruit were packaged without sugar or sugar sirup and five lots with 40 per cent sugar sirup in heat-sealable, cellophane-lined pint containers. Four hundred and six grams was the average weight of grapefruit used for the plain-packed fruit and 397 grams of grapefruit with 80 milliliters of 40 per cent sugar sirup for the sirup-packed fruit. This procedure was repeated four times. One hundred grams of fresh grapefruit and one-fourth of the solid and liquid contents of each container which had been allowed to thaw in the refrigerator overnight were weighed out for ascorbic acid determinations. These were done by the titration method, previously described, after approximately 24 hours in the freezing unit and thawing, and after freezing followed by one, three, six, and nine months of freezer storage and thawing.

Four lots of strawberries of the Blakemore variety were purchased on the market in May, 1947. The berries were washed, hulled, and puréed through a conical aluminum sieve with a wooden masher. The purée was mixed with sugar in a five-to-one and a three-to-one ratio, and the mixtures packed in cellophane-lined pint containers which were heat-sealed, or in glass jars designed for freezer storage. Reduced ascorbic acid was determined by the formaldehyde modification of the Robinson and Stotz (1945) method. Seventy-five-gram samples of fresh strawberry purée, before the addition of sugar, and of the thawed, frozen purée after one, three, and six months of freezer storage, were used for the determinations.

All the samples with the exception of the muskmelon processed in 1946 were frozen in a freezing unit of the plate type. Temperatures on the plates ranged from —24 to —30°C.(—11.2 to —22°F.). After approximately 24 hours on the plates the containers were transferred to freezer storage at a temperature of —17°C. ± 2.8°(1.4°F. ± 5°). In 1946 the muskmelon was frozen and stored in a home freezer where the temperature was —17.8°C. ± 1.7°(0°F. ± 3°) in both the freezing and storage compartments.

RESULTS AND DISCUSSION

Since three methods were used to determine the amounts of ascorbic acid present in the fruits studied, comparative figures obtained by these

methods for a few fruits and vegetables are presented (Table 1). It is apparent that the differences, if any, between the means are small and well within the limits of experimental error.

TABLE 1

Reduced Ascorbic Acid Determined by Three Methods

Product	Condition of product	Number of determinations	Ascorbic acid mean and average deviation of the mean determined by three methods		
			Titration	Loeffler and Ponting	Robinson and Stotz
			mg./gm.	*mg./gm.*	*mg./gm.*
Broccoli—Italian, green sprouting	Frozen 6 months	8	.66±.06	.68±.03	.65±.06
Grapefruit section	Fresh	2	.38±.00	.36±.01	.38±.00
Lima beans—Illinois large podded	Frozen 1 month	4	.22±.01	.21±.00	.22±.01[2]
Muskmelon	Frozen 6 months	4	.....[1]	.25±.04	.26±.02
Orange juice	Fresh	2	.51±.00	.52±.02	.52±.00
Orange juice	Canned concentrated	2	.65±.00	.66±.00	.66±.00

[1] Not determined by the titration method. [2] Formaldehyde modification was necessary.

Values for the reduced ascorbic acid content of freshly harvested Bender's Surprise and Hale's Best muskmelon and of these varieties of muskmelon, after 24 hours in the freezing unit and after one, three, six, and nine months of freezer storage are given (Table 2). The mean amount of ascorbic acid present in the Bender's Surprise muskmelon used was 0.19 milligram per gram which is slightly lower than the 0.23 milligram per gram reported by Wheeler *et al.* (1939) or the value of 0.24 milligram per gram obtained by Wagner *et al.* (1940). Hale's Best muskmelon contained a significantly [1] greater amount of ascorbic acid, 0.30 milligram per gram of freshly picked fruit. This figure is within the range of the values reported in the literature for the same variety. Floyd and Fraps (1939), Moseley and Satterfield (1940), and Wagner *et al.* (1940), found 33.3 and 25.47 milligrams per 100 grams and 0.38 milligram per gram, respectively.

During 24 hours in the freezing unit and thawing overnight in the refrigerator there were significant losses in the reduced ascorbic acid contents of plain-packed Bender's Surprise and Hale's Best muskmelon. After nine months of freezer storage the retention of ascorbic acid in the plain-packed Bender's Surprise muskmelon decreased from 26 to 12 per cent; in the Hale's Best muskmelon the decrease was from 37 to 18 per cent. The difference in the former case is not statistically significant, while it is in the latter. There were significantly greater retentions of ascorbic acid in the fruit packed for freezing in 40 per cent sugar sirup. Bender's

[1] A difference is considered significant if it is three or more times the standard error of that difference.

TABLE 2

Reduced Ascorbic Acid Content of Muskmelon Within Two Hours of Harvesting, After 24 Hours in the Freezing Unit, and After One, Three, Six, and Nine Months of Freezer Storage

Variety and type of pack	Fresh		After 24 hours in the freezing unit and thawing		After one month of freezer storage and thawing		After three months of freezer storage and thawing		After six months of freezer storage and thawing		After nine months of freezer storage and thawing	
	Moisture mean and standard deviation of the mean	Ascorbic acid mean and standard deviation of the mean	Mean amount of ascorbic acid per pint container	Retention of ascorbic acid mean and standard deviation of the mean	Mean amount of ascorbic acid per pint container	Retention of ascorbic acid mean and standard deviation of the mean	Mean amount of ascorbic acid per pint container	Retention of ascorbic acid mean and standard deviation of the mean	Mean amount of ascorbic acid per pint container	Retention of ascorbic acid mean and standard deviation of the mean	Mean amount of ascorbic acid per pint container	Retention of ascorbic acid mean and standard deviation of the mean
	pct.	*mg./gm.*	*mg.*	*pct.*	*mg.*	*pct.*	*mg.*	*pct.*	*mg.*	*pct.*	*mg.*	*pct.*
Bender's Surprise without sugar	93.4±.20	.19±.015	*15.3	26± 4.6	13.6	22±5.5	12.0	21±3.6	8.3	14±4.5	7.6	12± 4.4
Bender's Surprise with 40 per cent sugar sirup	93.4±.20	.19±.015	43.7	77±12.3	41.7	71±5.7	42.3	72±3.8	35.3	60±3.8	30.9	50±12.8
Hale's Best without sugar	92.1±.26	.30±.013	33.7	37± 2.5	32.0	34±4.4	20.8	23±3.7	25.0	27±3.8	16.3	18± 3.0
Hale's Best with 40 per cent sugar sirup	92.1±.26	.30±.013	77.9	86±11.2	85.8	93±6.6	83.4	91±5.8	69.9	76±7.8	57.9	63± 7.2

Surprise and Hale's Best varieties retained 77 and 86 per cents, respectively, of the amounts originally present after 24 hours of freezing and after thawing; these amounts decreased to 50 and 63 per cents, respectively, after nine months of freezer storage. As the standard deviations indicate, there were wide variations in results from lot to lot and neither of these losses is statistically significant.

Figures for the reduced and total ascorbic acid contents of muskmelon purchased on the market and then treated similarly to the known varieties of melon investigated the previous year are presented (Table 3). The original amount of ascorbic acid present, 0.40 milligram per gram, and the amounts retained after freezing, freezer storage, and thawing are higher for this unknown variety. There were no significant losses of ascorbic acid in either pack during the nine months of freezer storage. The muskmelon packed in 40 per cent sugar sirup retained from 90 to 96 per cent of the reduced and total ascorbic acid throughout the period of the experiment, significantly greater retentions than the 36 to 60 per cent obtained in the plain-packed muskmelon. A comparison of the mean values for the reduced and total ascorbic acid contents indicates that in the plain-packed fruit some of the original reduced ascorbic acid exists in the dehydro form after freezing, freezer storage, and thawing. In the sirup-packed fruit both the amounts and retentions of the reduced and total ascorbic acid are very similar and the presence of any dehydroascorbic acid is questionable.

Evidence that the reduced ascorbic acid present in grapefruit is stable during freezing, freezer storage, and thawing is given (Table 4). The mean amount of ascorbic acid present in the fresh grapefruit sections was 0.37 milligram per gram, which is comparable to the values reported by other workers. When the sections were frozen plain and in 40 per cent sugar sirup, there were no significant losses of ascorbic acid during 24 hours in the freezing unit followed by thawing in the refrigerator. Percentage retentions of reduced ascorbic acid varied from 93 to 102 during the nine months of freezer storage.

Although subjective ratings of the appearance, color, texture, and flavor of thawed, frozen muskmelon and grapefruit, prepared for freezing in five different ways, were obtained as part of an earlier study, it seems pertinent to consider here the palatability of the plain- and sirup-packed fruit. "Bite-size" wedges of melon and sections of white grapefruit, which had been frozen in 40 per cent sugar sirup, were rated as definitely superior to the plain-packed fruit. The thawed, frozen products which had been frozen in sugar sirup retained their natural appearance and colors and had good flavors. The texture of the muskmelon was similar to the fresh, while the grapefruit sections were slightly limp.

Values for the reduced ascorbic acid content of strawberry purée after preparation and after one, three, and six months of freezer storage are presented (Table 5). Fresh strawberries of the Blakemore variety, purchased on the market and sampled after puréeing but before the addition of sugar, contained 0.36 $\pm$ .02 milligram of ascorbic acid per gram. This figure is low for strawberry varieties in general, but agrees well with the 32.6 milligrams per 100 grams for the same variety reported by Lineberry

TABLE 3

TABLE 3

Reduced and Total Ascorbic Acid Content of Muskmelon When Fresh, After Freezing 24 Hours, and After One, Three, Six, and Nine Months of Freezer Storage

	Fresh		After 24 hours in the freezing unit and thawing		After one month of freezer storage and thawing		After three months of freezer storage and thawing		After six months of freezer storage and thawing		After nine months of freezer storage and thawing	
	Moisture mean and standard deviation of the mean	Ascorbic acid mean and standard deviation of the mean	Mean amount of ascorbic acid per pint container	Retention of ascorbic acid mean and standard deviation of the mean	Mean amount of ascorbic acid per pint container	Retention of ascorbic acid mean and standard deviation of the mean	Mean amount of ascorbic acid per pint container	Retention of ascorbic acid mean and standard deviation of the mean	Mean amount of ascorbic acid per pint container	Retention of ascorbic acid mean and standard deviation of the mean	Mean amount of ascorbic acid per pint container	Retention of ascorbic acid mean and standard deviation of the mean
	pct.	*mg./gm.*	*mg.*	*pct.*	*mg.*	*pct.*	*mg.*	*pct.*	*mg.*	*pct.*	*mg.*	*pct.*
Reduced ascorbic acid in plain-packed muskmelon	89.2±.12	.40±.024	45.3	36±5.7	45.6	36±5.4	50.8	41±8.7	49.0	39±7.6	47.0	37±7.0
Total ascorbic acid in plain-packed muskmelon	89.2±.12	.42±.011	71.8	55±5.7	79.1	60±8.2	75.2	58±6.1	62.2	48±9.0	71.2	54±4.3
Reduced ascorbic acid in sirup-packed muskmelon	89.2±.12	.40±.024	113.8	92±2.6	116.3	94±3.8	111.6	90±5.2	119.8	96±5.0	116.2	91±4.0
Total ascorbic acid in sirup-packed muskmelon	89.2±.12	.42±.011	120.2	92±1.7	123.0	94±2.8	117.1	90±6.4	123.8	95±8.7	118.2	91±2.7

TABLE 5

Reduced Ascorbic Acid Content of Strawberry Purée When Fresh and After One, Three, and Six Months of Freezer Storage

Type of pack		Fresh		After one month of freezer storage and thawing			After three months of freezer storage and thawing			After six months of freezer storage and thawing		
Proportion of purée to sugar	Container	Moisture mean and standard deviation of the mean	Ascorbic acid mean and standard deviation of the mean sugar-free basis	Mean amount of ascorbic acid		Total retention of ascorbic acid mean and standard deviation of the mean	Mean amount of ascorbic acid		Total retention of ascorbic acid mean and standard deviation of the mean	Mean amount of ascorbic acid		Total retention of ascorbic acid mean and standard deviation of the mean
				As determined	Sugar-free basis		As determined	Sugar-free basis		As determined	Sugar-free basis	
		pct.	*mg./gm.*	*mg./gm.*	*mg./gm.*	*pct.*	*mg./gm.*	*mg./gm.*	*pct.*	*mg./gm.*	*mg./gm.*	*pct.*
5:1	Cellophane-lined carton	92.1±.37	.36±.021	.24	.29	81±2.4	.25	.30	84±3.4	.26	.30	84±2.6
5:1	Glass jar	92.1±.37	.36±.021	.24	.28	78±3.6	.25	.30	82±3.1	.25	.30	83±2.3
3:1	Glass jar	92.1±.37	.36±.021	.22	.29	81±2.0	.23	.30	84±2.3	.23	.31	86±4.5

and Burkhart (1942) and is only slightly lower than the 0.426 milligram per gram found by Satterfield and Yarbrough (1940).

When sampled after one month of freezer storage and defrosting overnight in the refrigerator, purées mixed with sugar in a five-to-one or three-to-one ratio and packed in glass freezer jars or moisture-vapor-proof cartons retained between 78 and 81 per cent of the ascorbic acid present after puréeing. There was no evidence of further loss of ascorbic acid during the following five months of freezer storage; the slight gains are not statistically significant. Furthermore, there was no significant difference between the amounts of ascorbic acid retained in purée packed in glass jars and in cellophane-lined containers; nor was there a difference in retention of ascorbic acid in purée mixed with sugar in the proportions of five-to-one and three-to-one. These results are not in agreement with those of Kirk and Tressler (1941), who found an inverse relationship between the amount of sugar added and the retention of ascorbic acid. However, they are within the range of values reported by Loeffler (1946).

TABLE 4

Reduced Ascorbic Acid Content of Fresh and Frozen Texas Grapefruit

Treatment	Grapefruit sections packed plain		Grapefruit sections packed with 40 per cent sugar sirup	
	Mean amount of ascorbic acid per pint container	Retention of ascorbic acid mean and standard deviation of the mean	Mean amount of ascorbic per pint container	Retention of ascorbic acid mean and standard deviation of the mean
	mg.	*pct.*	*mg.*	*pct.*
Fresh	148.3	.....	145.2	.....
Frozen 24 hours and thawed	147.2	99±1.6	144.8	100±2.7
Frozen one month and thawed	150.3	102±2.1	147.1	102±2.1
Frozen three months and thawed	139.9	94±2.0	141.7	98±2.6
Frozen six months and thawed	138.2	93±0.8	141.6	98±1.7
Frozen nine months and thawed	141.6	96±1.1	140.0	96±1.4

SUMMARY

Freshly harvested Bender's Surprise and Hale's Best muskmelon, packed for freezing without sugar or sugar sirup, retained 26 and 37 per cents, respectively, of the reduced ascorbic acid originally present after 24 hours in the freezing unit and thawing overnight in the refrigerator. Significantly larger percentages were retained when the same varieties were packed in 40 per cent sugar sirup—77 per cent ascorbic acid in Bender's Surprise, and 86 per cent in Hale's Best muskmelon. Further losses during nine months of freezer storage were not significant except in the case of the plain-packed Hale's Best muskmelon, where the ascorbic acid retention decreased from 37 to 18 per cent.

In muskmelon purchased on the market the per cent retentions of reduced ascorbic acid in the thawed, frozen samples ranged from 90 to 96 for the sirup-packed fruit and from 36 to 41 for the plain-packed fruit. After freezing and each interval of freezer storage there were signifi-

cantly greater amounts of reduced ascorbic acid in the sirup-packed fruit. Neither pack gave evidence of significant losses of ascorbic acid during nine months of freezer storage. The presence of dehydroascorbic acid in the plain-packed fruit is indicated by the greater amounts of total, rather than of reduced, ascorbic acid present, although the differences in percentage retentions are not statistically significant. In the sirup-packed fruit there were negligible amounts of dehydroascorbic acid.

No significant losses of reduced ascorbic acid occurred in grapefruit sections packed with or without 40 per cent sugar sirup during 24 hours in the freezing unit followed by thawing in the refrigerator. The percentage retentions after one to nine months of freezer storage ranged from 93 to 102 in the plain-packed fruit and 96 to 102 in the sirup-packed.

The muskmelon and grapefruit, packed for freezing in 40 per cent sugar sirup, were more palatable than the plain-packed fruits after freezing, intervals of freezer storage, and thawing.

Strawberries of the Blakemore variety, puréed, mixed with sugar in the proportion of five parts of purée to one of sugar or three parts of purée to one of sugar, and packed in cellophane-lined cartons or glass freezer jars, lost approximately 20 per cent of the reduced ascorbic acid content after freezing, one month of freezer storage, and thawing. No further losses occurred during three and six months of freezer storage. The two concentrations of sugar and the two types of containers used in this study had no significant effect on the retention of ascorbic acid.

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THERMAL PROCESSING OF CANNED FOODS IN TIN CONTAINERS. V. EFFECT OF RETORTING PROCEDURES ON STERILIZATION VALUES IN CANNED FOODS

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For successful preservation of a canned food by a thermal process, it must be subjected to heat for a sufficient time to produce a condition of commercial sterility. This heat treatment is called a process. Originally the determination of adequate processes had been by "cut and try" experimentation or by commercial experience with similar products. The problem of process determination was approached on a scientific basis almost 30 years ago by Bigelow, Bohart, Richardson, and Ball (1920) and their method of process calculation was simplified by the method of Ball (1928) and further by the modified methods of Olson and Stevens (1939) or Schultz and Olson (1940). In the study of process times, the rate of heat penetration into the canned food is measured by use of a thermocouple by the method described by Pilcher *et al.* (1943). Obviously, determination of the process time and temperature can be made by one of these methods with heat penetration data obtained for the particular conditions under which the test was conducted. This process, however, will be strictly valid only for the conditions of the heat penetration test. Conditions to which the filled cans are subjected in various canneries between the time the cans are closed and the process is under way will vary considerably. It is important that these various factors that affect the rate of heat penetration be appreciated in order that they can be given their proper consideration in evaluation of the process. Investigations reported herein provide a basis for evaluation of the effect of (1) the temperature distribution within the container at the start of the process and (2) retort come-up time.

Effect of Temperature Distribution in Can Contents: When a conduction-heating product is filled into a can hot and allowed to cool in air for a period of time before processing, as must be practiced commercially when accumulating a retort load of cans, the resulting differential in temperature between the center of the can and the product near the can wall, will, according to Olson and Jackson (1942), have a marked effect on the *j* value where

j = The ratio of jI/I. The value of j is a measure of the lag in heating rate at the start of the heating period.

I = The difference, RT—IT.

RT = The retort temperature in °F.

IT = The temperature at which the heating curve intersects the axis of ordinates [initial temperature or temperature (°F.) at center of can at start of process].

jI = The difference between RT and the temperature at which the straight line portion of the heating curve intersects the axis of ordinates.

Olson and Jackson (1942) estimated, both theoretically and experimentally, the effect of different temperature distributions within the container on the value of *j*. In the present work the effect on the sterilizing value of treatments resulting in different temperature distributions was studied. These treatments involved different holding times in air between the filling and closing operations and the beginning of the process.

EXPERIMENTAL PROCEDURE

To determine the effect of temperature distribution within the container, on the subsequent rate of heat penetration during processing, heat penetration tests were made on a five per cent suspension of bentonite in water, prepared by thoroughly mixing the 300-mesh bentonite with water in a food blender. This suspension is a close approximation to conduction-heating food products, insofar as the heat penetration rate is concerned. Measurement of the rate of heat penetration into the bentonite suspensions in No. 2 (307 x 409) and No. 10 (603 x 700) size cans was accomplished by use of special copper-constantan thermocouples inserted through fittings in the container walls and having the hot junctions located at the geometric centers of the cans. The filled cans were sealed under vacuum at room temperature and later processed in a vertical position in a small retort.

In order to simulate conditions in which containers are filled with a heated product, the filled and sealed cans were heated and shaken thoroughly to equalize the temperature of the suspension. These cans were then allowed to air-cool for periods of time typical of the maximum holding time for several canning-line operating speeds. Because heat was lost from the can walls, a temperature differential was established within the container. After the desired holding period, a part of the cans were processed without disturbance of the contents. Additional cans were shaken to obtain a uniform temperature distribution just before processing, to be used as a basis for calculating the effect of temperature differences of the contents in unshaken cans. The heat penetration factors, f_h and j, were determined from heating data obtained by means described by Ball (1928). The value f_h is the number of minutes required for the straight line portion of the heating curve to traverse one logarithmic cycle when plotted on semi-log paper.

Sterilizing value (F_o) effective at the can center were used as a basis of comparison to test the effect of the variables. These sterilizing values were calculated by the method of Ball (1928) and were based on the process of 80 minutes at 116°C.(240°F.) for No. 2 cans and a process of 180 minutes at 116°C. for No. 10 cans.

RESULTS

Effects of the cooling during holding in air are reflected in increase in *j* values as shown (Table 1). Cans listed as shaken before processing represent a condition of uniform temperature distribution at the time

the process is started. Therefore, the effect of the holding time on the sterilizing value is shown by comparing the paired F_o values (Column B, Table 1). The F_o values are significantly reduced when the hot filled cans are held for any period of time after closing and before processing. One F_o value was obtained by taking the heat penetration data from the shaken can and assuming an initial temperature equal to the filling temperature in evaluating the processes. The other F_o value was calculated on the basis of actual data from the unshaken can. Although there is no correlation indicated between holding time and loss of sterilizing value, there is a very significant loss in sterilizing value for holding times as short as 15 minutes.

F_o values (Column A, Table 1) illustrate errors that can be introduced by using heat penetration data from a can processed immediately after filling to evaluate a process for a can held for various intervals of time before processing. The maximum error is 100 per cent in the case of Cans *m* and *n*, representing a No. 10 can held for 50 minutes before processing. The No. 2 cans do not show this great an effect; a 60-minute hold (Cans *i* and *j*) shows a reduction in sterilizing value of only 0.5 F_o unit, or 10.4 per cent.

Calculations made on the same basis as those for Column B, Table 1, show the increase in process necessary for the can center temperature to reach within one degree of retort temperature (which gives equivalent F_o values) for cans held for a period of time after closing, as compared with a can processed immediately after sealing. These figures are shown (Column C, Table 1).

Shaking the cans to produce a uniform temperature distribution before processing did not result in a significant change in sterilizing value. The faster heat penetration was compensated by the lower initial temperature, the net result being little or no change in sterilizing value. In some cases the sterilizing value appears to be lower for the shaken cans. This is shown (Column D, Table 1).

DISCUSSION

It is established that for a conduction-heating product, extending the time interval between closing and processing, results in a reduction in rate of heat penetration and hence in the effective sterilizing value of the process. This is not necessarily a result of a lower can center temperature at the beginning of the process, because the effect is noted even when no change in this temperature occurs. Mixing the can contents by shaking the container after a holding period produces a lower initial temperature, but the heat penetration is faster, the net result being little or no change in the effective sterilizing value of the process. Possibility of a significant error in process evaluation suggests itself if heat penetration measurements are made under a different holding period condition than commercially processed cans. The obvious solution, making the heat penetration test under holding conditions comparable to commercially packed cans, is not always feasible because of the possibility of wide variation in handling commercially packed cans. It would appear preferable to standardize heat penetration technique so that at the beginning of the process the temperature is uniform throughout the can. Data from such tests can then be

TABLE 1

Effect of Distribution of Temperature of Can Contents at Beginning of a Process on Its Sterilizing Value for a Conduction-Heating Product

Can	Can size	Filling temp.	Time of hold	Can shaken before process	Can center temp. start of process	j	f_h	A		B		C			D	
								Process IT/B_R/RT	F_o	Process IT/B_R/RT	F_o	IT	Time to 239°F.	Diff.	Process IT/B_R/RT	F_o
	No.	*°F.*	*min.*		*°F.*			*°F./min./°F.*		*°F./min./°F.*		*°F.*	*min.*	*m.n.*	*°F./min./°F.*	
a	2	205	40	No	185	1.964	53.6	180/ 80/240	4.4	185/ 80/240	4.8	185	109			
b	2	183	0	Yes	183	1.585	53.6	180/ 80/240	5.4	205/ 80/240	8.0	205	94	15		
c	2	189	15	No	186	2.030	54.7	180/ 80/240	4.1	186/ 80/240	4.5	186	112		186/ 80/240	4.5
d	2	187	15	Yes	172	1.662	54.7	180/ 80/240	5.0	189/ 80/240	5.7	189	105	7	172/ 80/240	4.4
e	2	189	30	No	183	1.842	54.7	180/ 80/240	4.5	183/ 80/240	4.7	183	110		183/ 80/240	4.7
f	2	189	30	Yes	169	1.605	54.7	180/ 80/240	5.1	189/ 80/240	5.3	189	105	5	169/ 80/240	4.4
g	2	185	30	No	172	2.100	52.2	180/ 80/240	4.4	172/ 80/240	3.9	172	112		172/ 80/240	3.9
h	2	184	30	Yes	162	1.830	52.2	180/ 80/240	5.0	185/ 80/240	5.4	185	105	7	162/ 80/240	3.9
i	2	182	60	No	154	2.140	52.2	180/ 80/240	4.3	154/ 80/240	2.9	154	118		154/ 80/240	2.9
j	2	184	60	Yes	144	1.920	52.2	180/ 80/240	4.8	182/ 80/240	5.0	182	107	11	144/ 80/240	2.9
k	10	198	30	No	198	2.380	167.0	180/180/240	2.8	198/180/240	5.3	198	334			
l	10	197	0	Yes	197	1.768	167.0	180/180/240	4.8	198/180/240	8.0	198	312	22		
m	10	204	50	No	199	2.180	191.0	180/180/240	1.9	199/180/240	4.3	199	372		199/180/240	4.3
n	10	206	50	Yes	168	1.595	191.0	180/180/240	3.8	204/180/240	8.5	204	336	36	168/180/240	2.6
o	10	167	60	No	167	2.055	174.5	180/180/240	3.0	167/180/240	2.0	167	380		167/180/240	2.0
p	10	169	60	Yes	150	1.733	174.5	180/180/240	4.3	167/180/240	2.9	167	367	13	150/180/240	1.8
q	10	174	120	No	161	2.075	174.5	180/180/240	3.0	161/180/240	1.6	161	387		161/180/240	1.6
r	10	171	120	Yes	141	1.820	174.5	180/180/240	3.9	174/180/240	3.2	174	363	24	141/180/240	1.3

applied to any processing condition, providing process time is based on an initial temperature corresponding to the average temperature of the can contents rather than the can center temperature at the start of the process. F_o data (Column D, Table 1) show that substantially, the same F_o values are obtained when this technique is used as when actual heat penetration and initial temperature data are used in the calculations.

Effect of Variations in Retort Come-Up Time: In the thermal processing of canned foods in still retorts a certain period of time is required to bring the retort to processing temperature after the steam has been turned on. Heat to which the can is exposed during this "come-up" will be less effective than the final retort temperature, but will shorten the length of time necessary to process the can of food by some fraction of the time consumed in bringing the retort to the processing temperature.

In development of the formula method for process determinations, Ball (1928) has taken into account the effect of varying the time taken to bring the retort to processing temperature. The value of the come-up period in terms of process time was found by Ball to be 42 per cent of the come-up time. In arriving at this figure, however, a uniform rate of increase in the temperature of the retort was used and the products involved were of such consistency that heating within the container took place by conduction only. Consequently, a further investigation was undertaken which included different rates of temperature rise in the retort, various can sizes, and convection-heating products compared to conduction-heating products.

The form of the come-up curves found in actual practice in commercial canneries varied considerably, some following a straight line on semi-logarithmic paper (logarithmic temperature scale), some being very nearly a straight line on coordinate paper, while others were quite erratic.

EXPERIMENTAL PROCEDURE

In the first tests made in this study, heating tests were conducted in cans filled with cream-style corn. In later tests, suspensions of bentonite in water were used, since it has been found by Jackson and Olson (1940) that a given suspension can be reheated several times without appreciable change in the heating characteristics. For studies of a conduction-heating product, a five per cent bentonite suspension was used, and for convection-heating tests a two per cent suspension.

The filled and sealed No. 2 cans were processed in a retort, the temperature of which was manually controlled during the come-up time to follow the type of curve desired. Come-up times of 20 and six minutes were used with conduction-heating products. The longer time was used in order to reduce experimental error, since small differences in time would not affect the percentage calculations to as great a degree as would be found with shorter come-up times. With convection-heating products, however, the shorter period of come-up time was used. As a basis for calculations, heating tests were also conducted with "zero come-up time." The retort in these tests was brought to processing temperature in slightly over 30 seconds.

Data obtained from various heat penetration tests were plotted on semi-log paper and heat penetration factors determined, the value j being taken

from "steam-on" time. Process times (B_B) were calculated for $g = 1$ for conduction heating and $g = 0.1$ for convection heating from the following equation:

$$B_B = f_h \log \frac{(jI)}{(g)}$$

Cans having quivalent g and f_h values have equivalent sterilizing values (F_o) where g is the number of degrees below retort temperature on the heating curve at the end of the heating period.

The percentage sterilizing value (X) of the come-up time was calculated by the following equation:

$$X \text{ per cent} = \frac{T - (B_{B_1} - B_{B_2})\ 100}{T}$$

where T = come-up time, B_{B_1} = calculated process time measured from steam-up time when the come-up time is greater than zero, and B_{B_2} = process time when the come-up time is zero. Data obtained and calculated results are given (Table 2).

RESULTS

With conduction-heating products in No. 2 size cans, a 20-minute come-up with a uniform temperature rise results in an average come-up correction factor of 37.5 per cent, which is comparatively close to the figure 42 per cent suggested by Ball. Rather wide deviations from this figure were found in individual tests, however. When the come-up follows a semi-logarithmic curve, the percentage sterilizing value of the come-up time is considerably higher, roughly 70 per cent.

With convection- and conduction-heating products using a uniform rate of temperature rise during the come-up period of six minutes, results were very erratic, the come-up correction factor showing a variation from 18 to 65 per cent, with an average of 34 per cent.

In view of the variation in the shape of the come-up curves found in commercial practice, and since the sterilizing value of the come-up time is lowest when the retort temperature rises uniformly, it appears that the practice of using the 42 per cent figure, as derived by Ball, warrants continuation.

SUMMARY

Studies indicate that in the processing of canned foods the process used should be based on an "initial temperature," which is the average temperature of the can contents at the start of the process, rather than the can center temperature, in order to compensate for the effect of cooling of cans between closing and processing.

In order to improve accuracy in calculating processes, variations in temperature distributions in the can at the beginning of the process in heat penetration tests should be controlled or measured.

Studies were made to determine the effective heating value of the retort come-up time in the processing of canned foods when the rate of temperature increase was varied and using products heating both by convection and conduction. Results indicated that the value of the come-up period

TABLE 2

Effect of Retort Come-Up on Theoretical Process Time

TABLE 2

Effect of Retort Come-Up on Theoretical Process Time

Product	Come-Up Time	Come-Up Rate[1]	Retort Temp.	I.T.	f_h	j	g	B_B	Increase	X
	min.		*°F.*	*°F.*					*min.*	*pct.*
Cream-style corn	0	I	250	70	60.7	2.020	1	155.50		
	20	L	250	70	60.7	2.388	1	159.90	4.40	78.0
	20	U	250	70	60.7	2.956	1	165.50	10.00	50.0
Cream-style corn	0	I	240	70	61.8	1.908	1	153.60		
	20	L	240	70	61.8	2.270	1	158.20	4.60	77.0
	20	U	240	70	61.8	2.944	1	165.20	11.60	42.0
Cream-style corn	0	I	230	70	63.7	1.666	1	156.20		
	20	L	230	70	63.7	2.170	1	163.50	7.30	63.5
	20	U	230	70	63.7	2.499	1	167.40	11.20	44.0
5 per cent bentonite	0	I	250	70	53.4	1.824	1	134.40		
	20	L	250	70	53.4	2.476	1	141.40	7.00	65.0
	20	U	250	70	53.4	3.498	1	149.40	15.00	25.0
5 per cent bentonite	0	I	240	70	56.1	1.810	1	139.50		
	20	L	240	70	56.1	2.174	1	144.00	4.50	77.5
	20	U	240	70	56.1	2.890	1	151.00	11.50	42.5
5 per cent bentonite	0	I	230	70	53.0	1.795	1	130.20		
	20	L	230	70	53.0	2.563	1	138.50	8.30	58.5
	20	U	230	70	53.0	3.522	1	145.80	15.60	22.0
2 per cent bentonite	0	I	250	70	4.53	0.670	.1	13.96		
	6	U	250	70	4.53	5.520	.1	18.10	4.14	31.0
2 per cent bentonite	0	I	240	70	4.58	0.690	.1	14.05		
	6	U	240	70	4.57	6.650	.1	18.55	4.50	25.0
2 per cent bentonite	0	I	230	70	4.40	0.760	.1	13.59		
	6	U	230	70	4.40	10.060	.1	18.50	4.91	18.2
5 per cent bentonite	0	I	250	70	52.7	2.090	.1	188.30		
	6	U	250	70	52.7	2.272	.1	190.40	2.10	65.0
5 per cent bentonite	0	I	240	70	53.0	2.000	.1	187.10		
	6	U	240	70	53.0	2.470	.1	192.00	4.90	18.3
5 per cent bentonite	0	I	230	70	53.0	2.040	.1	186.10		
	6	U	230	70	53.0	2.465	.1	190.60	4.50	25.0

[1] I—Instantaneous or "zero" come-up time—actually about 30 seconds; L—semi-logarithmic rate of temperature increase; U—uniform rate of temperature rise. See definitions of terms for explanation of column headings.

in value of process time should be accepted as 42 per cent of the come-up time as found by Ball (1928). This figure should be used for both convection- and conduction-heating products and for logarithmic and uniform rates of temperature rise during the come-up period.

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STABILITY OF COLOR IN FRUIT JUICES[1]

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The present investigation was undertaken to study the effects of various factors on the stability of color in a variety of fruit juices during storage, and to observe the effects of such factors on the color of anthocyanin pigments isolated from some of the fresh fruits.

The agents responsible for deterioration of color in fruit products were summarized by Joslyn (1941). He pointed out that the problem of color retention in fruit products is complicated by the multiplicity of reactions which may be involved in bleaching, discoloration, and browning. The natural plant pigments may change in tint or hue during preparation, processing, or storage of the fruit products. Browning may occur through formation of pigmented substances resulting from decomposition of certain chemical constituents, or by reactions of some of the constituents of the fruit with oxygen. Browning may also result from reactions between constituents naturally present or introduced in manufacture.

Pederson, Beattie, and Stotz (1947) have indicated that changes which occur in fruit juices during storage are very complex in nature. Though enzymes are apparently inactivated, and effects of oxygen, light, and metals are reduced to a minimum, serious deteriorative changes nevertheless occur. Pederson (1947) attributed these changes to chemical reactions between various ingredients of the juice.

Studies of Tressler and Pederson (1936) and Pederson, Beattie, and Beavens (1941) showed that a progressive darkening in the color of strawberry, currant, and raspberry juices occurred during storage at higher temperatures. This was most noticeable with strawberry juice. The color changed from a bright, clear red to a dull red, and finally to brown. They also noted that color changes occurring in the juices could be partially overcome by storage at low temperatures. These observations have been more recently confirmed by Pederson (1947).

In an investigation of the color changes in fruit spreads, Kertesz (1948) observed that color loss in strawberry spread was greatly accelerated at temperatures between 18.3 and 21.1°C.(65 and 70°F.). He noted that very little loss of color occurred in these spreads during storage at 1.1°C.(34°F.) for several months, but that changes proceeded at an easily measurable rate at 15.6°C.(60°F.), and much faster at 37.8°C. (100°F.). More recent studies by Kertesz and Sondheimer (1948b) indicated that color loss in strawberry preserves due to high temperature may be lessened by greater care in cooking, immediate cooling below

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15.6°C.(60°F.), storage below 15.6°C.(60°F.), and refrigeration when product is held for long periods.

Tressler and Pederson (1936) found that pasteurized grape juice, packed in a high vacuum or in bottles containing substantially no oxygen, underwent very little deterioration, even when exposed to light at room temperature. On the other hand, juices in partially filled bottles deteriorated rapidly. Changes noted were a clouding of the juice, and a change from bright purple-red to brown. A slow deposition of brown sediment occurred, accompanied by detrimental changes in aroma and flavor. These changes occurred more rapidly at room temperature than at lower temperatures. These authors also noted that air in the headspace of bottled strawberry juice resulted in increased deterioration of color.

Powers and Esselen (1942) indicated that effects of light were of only minor importance in the deterioration of glass-packed foods in comparison with deteriorative changes caused by heat and oxygen. Studies by these workers revealed that many undesirable changes attributed to light were actually caused by heat and oxygen, and that the effects of light were negligible. Under extreme conditions of exposure, light functioned as an accelerating agent, and speeded up, slightly, reactions which occurred even in the absence of light.

Beattie, Wheeler, and Pederson (1943) observed that in certain fruit juices, changes in color occurred concurrently with progressive losses of ascorbic acid during storage. They further noted that when ascorbic acid was added as an antioxidant to strawberry, currant, and raspberry juices, deterioration of color was accelerated. Siemers (1946) indicated that ascorbic acid, although effective in retarding discoloration and development of oxidative off-flavors in some fruit products, could not be considered to be of antioxidant value in retarding such changes in all fruit and vegetable products. Products such as strawberries, citrus, and tomatoes, which normally possess a relatively high ascorbic acid content, appear to derive little benefit from added ascorbic acid.

Powers, Esselen, and Fellers (1943) and Powers and Fellers (1945) showed the addition of ascorbic acid prevented or retarded development of discoloration and associated off-flavors of certain foods such as pears, peaches, and plums.

Esselen, Powers, and Woodward (1945) reported that the addition of 30 mg. of d-isoascorbic acid per pint of tomato juice was effective in retarding flavor loss and discoloration during storage under adverse conditions. In studies on the fortification of fruit juices with ascorbic acid, Esselen, Powers, and Fellers (1946) ascertained that color and flavor of grape juice were not improved to any great extent by addition of l-ascorbic acid.

EXPERIMENTAL PROCEDURE

This investigation was undertaken to study the effect of such factors as time and temperature of storage, and the relationship of oxygen, light, sugar, pH, and antioxidants on deteriorative changes in the color of seven representative fruit juices and strawberry fountain syrup. A second aspect dealt with the effect of these same factors on the color of the

anthocyanin pigments directly isolated from strawberries and currants.

Preparation of Experimental Packs: Experimental packs of blueberry, black cherry, currant, grape, red raspberry, strawberry, and tomato juices were prepared, processed, and stored under similar conditions. The grape juice was prepared as described by Chenoweth (1930). Sugar at the rate of three ounces per quart was added, except to that portion of the juice used for studies of juices prepared without the addition of sugar. For studies on the effect of pH values, the pH was adjusted by the addition of a 10 per cent solution of citric acid or sodium bicarbonate. The end points were selected to include a pH range between 0.5 and 0.75 unit above and below the original pH value of the respective juices (Table 2). This study was made to determine whether changes in pH influenced the stability of color of the different juices. For studies on the effect of oxygen, one portion of the juice was aerated by mixing in a Waring Blendor for five minutes, and another portion deaerated according to the method of Mottern and von Loesecke (1933). To one lot, ascorbic acid was added at the rate of 50 mg. per 100 ml. of juice. The various lots of juices were then filled into preheated one-half-pint fruit jars, partially sealed, and processed at 76.7°C.(170°F.) for 15 minutes. The jars were removed and completely sealed, and cooled under a fine cold-water spray.

Juices from other fruits—strawberry, red raspberry, black cherry, currant, and blueberry—were prepared by the heat extraction method. Water was added in the proportion of one cup to five pounds of fruit and the berries heated to 87.8°C.(190°F.) for two minutes. The heated berries were strained through two layers of cheesecloth, then through four layers of cheesecloth in order to further clarify the juice. Sugar was added to bring the soluble solids to approximately 15 per cent as measured by the refractometer. The finished juices were then divided into appropriate lots and treated in a manner similar to that of the grape juice.

Tomato juice was prepared by heating washed and cut tomatoes to approximately 71.1°C.(160°F.) for two minutes in a stainless steel kettle. The heated tomatoes were strained through two layers of cheesecloth, then through four layers of cheesecloth to completely remove any skin or seed particles that might have been present. The juice was heated to 82.2°C. (180°F.) and salt added at the rate of one ounce per gallon of juice. The juice was then divided into appropriate lots and filled hot into preheated one-half-pint fruit jars, partially sealed, and processed at 100°C.(212°F.) for 15 minutes. The jars were removed, completely sealed, and cooled under a fine cold-water spray.

For comparative studies on the effect of sugar, one lot from the experimental packs of each juice, with the exception of tomato juice, was put up without the addition of sugar. For similar studies on tomato juice, one lot was prepared with the addition of sugar, at the rate of four ounces per quart of juice.

Packs of strawberry fountain syrup were also studied to determine the effects of varying amounts of sugar and citric acid on the stability of color during storage at room temperature. This pack was divided into three

lots. The first lot was divided into five portions, each varying in concentration of sugar as follows:

Portion A—5 parts strawberry to 1 part sugar
Portion B—4 parts strawberry to 1 part sugar
Portion C—3 parts strawberry to 1 part sugar
Portion D—2 parts strawberry to 1 part sugar
Portion E—1 part strawberry to 1 part sugar

Lot One was prepared by mixing washed, cleaned, sliced strawberries with varying concentrations of sugar as specified above, and allowing the mixture to stand for two hours, to permit penetration of the sugar into the fruit. The strawberries were then heated to 87.8°C.(190°F.), and filled into preheated one-half-pint jars. The jars were partially sealed and processed in a hot-water bath at 100°C.(212°F.) for 15 minutes. The jars were then removed, completely sealed, and cooled under a fine cold-water spray. The second and third lots were prepared similarly, except they contained 0.5 and 0.25 per cent, respectively, by weight of citric acid which was added to the strawberries as a mixture with the sugar.

Conditions of Storage: Each pack of processed fruit juice was divided into groups of six jars each, representing the various experimental factors and conditions of storage. Storage studies covered a period of six months under the following conditions:

Temperature—Jars stored at 37.8°C.(100°F.); 21.1 to 26.7°C.(70 to 80°F.); 1.7°C.(35°F.); and —17.8°C.(0°F.) in the dark.

Light—Jars stored at 21.1 to 26.7°C.(70 to 80°F.) in daylight.

Methods of Analysis: Color changes in the juices were determined by measurement of light transmittance characteristics, using a Coleman Model 11 Spectrophotometer. Absorption maxima were determined for each juice, and subsequent measurements made at these determined wavelengths. Wavelengths used were 500 mμ. for strawberry, raspberry, grape, cherry, and blueberry juices; 450 mμ. for currant juice and strawberry fountain syrup; 475 mμ. for tomato juice (Figs. 1 and 2). The juices, with the exception of tomato juice, were analyzed as composite samples. Aliquot portions were taken, centrifuged for 10 minutes at 5,000 r.p.m., and the clear liquid decanted off. Before use the centrifuged juices were diluted to appropriate volume with distilled water so that light transmittance characteristics could be read from a sensitive portion of the galvanometer. Strawberry, raspberry, and currant juices were diluted one part in two parts of water, and blueberry, cherry, and grape juices one part in three parts of water. The Null point method of measurement was used, with water as the reference solution for the blank; high readings indicate light-colored juices, low readings darker-colored juices.

Tomato juice was prepared for spectrophotometric analysis by a modification of the method of Kramer and Smith (1946). A 10-ml. sample of the juice, to which was added 70 ml. of benzol, was transferred to a Waring Blendor, and blended for five minutes. The sample was next transferred to a 100-ml. graduated flask, and the solids allowed to settle on the bottom. A five-ml. aliquot was taken, diluted with 20 ml. of benzol,

and centrifuged for five minutes at 5,000 r.p.m. Transmission measurements were made with the centrifuged sample, using benzol as the reference solution for the blank.

The strawberry fountain syrup was first well mixed and the pieces of fruit thoroughly crushed. It was then prepared in a manner similar to strawberry juice for spectrophotometric analysis.

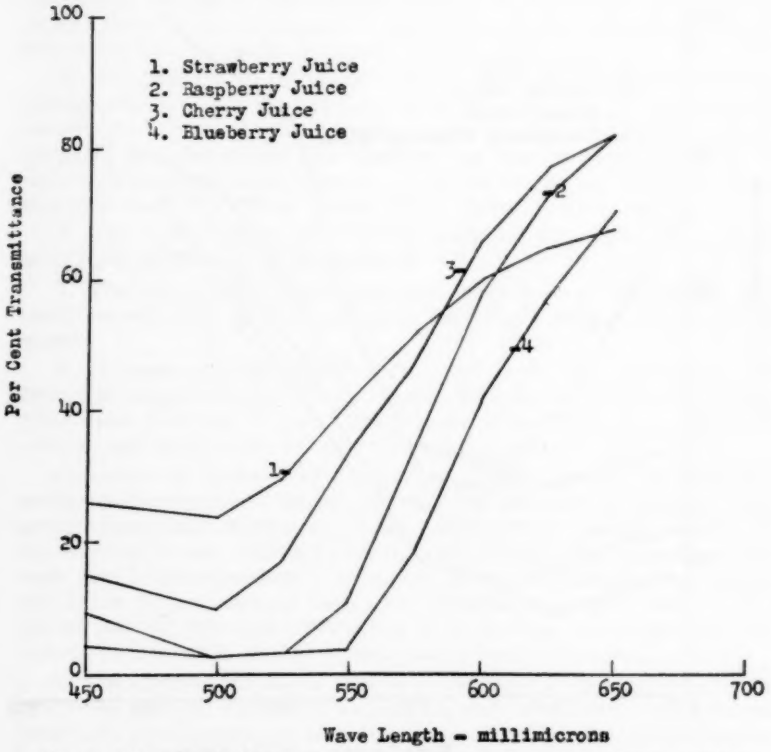

FIG. 1. Light transmittance values on control samples of strawberry, raspberry, cherry, and blueberry juices.

Visual Examination: To obtain a general estimate of acceptability of the juices in regard to color, samples were judged visually for obvious changes in appearance. Estimates of changes were based on comparison with similar samples stored at 1.7°C.(35°F.).

Anthocyanin Pigments: Anthocyanins are known to be present in fruits mainly in combination with sugars (usually glucose, rhamnose, galactose, or a combination of two sugars) as glucosides. As long as they are in this form, they are water soluble. On boiling with acid, however, the sugar is removed, leaving an anthocyanidin (a nonglucoside) insoluble in water. The intention of the present study was to obtain the antho-

cyanin pigment in relatively pure form as a glucoside to use for experimental purposes.

The red anthocyanin pigment of strawberry was recently isolated by Kertesz and Sondheimer (1948a) and identified as pelaronidin 3-monoglucoside. In the present investigation the anthocyanin pigment was isolated from strawberries and currants by chromatographic adsorption of crude

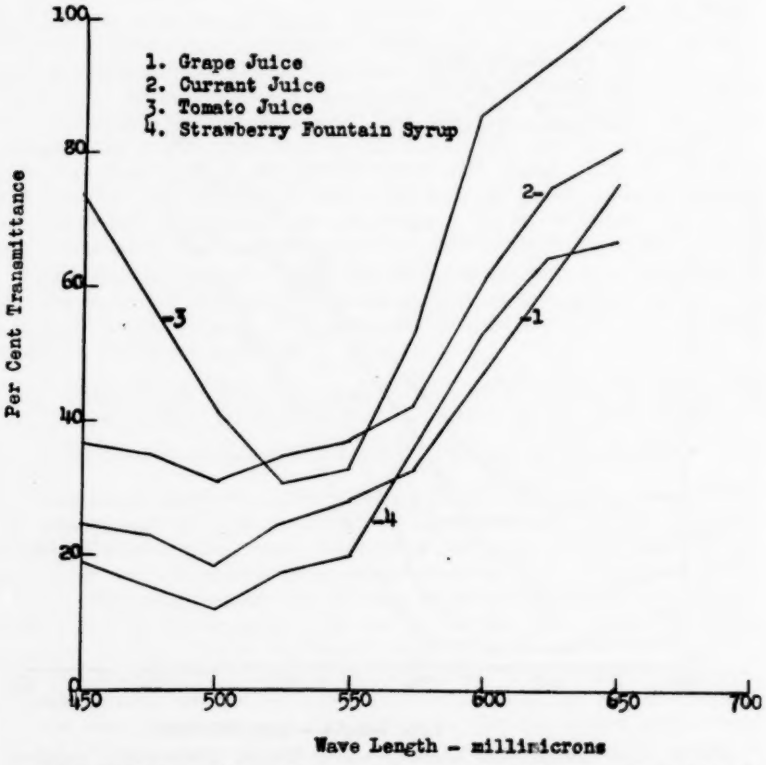

FIG. 2. Light transmittance values on control samples of currant, grape, and tomato juice and strawberry fountain syrup.

extracts of the pigment. This method consisted essentially of extraction of the pigment in crude-water extracts, purified by chromatographic adsorption. Procedures for each of the fruits are outlined in detail below.

Procedure for Isolation of Anthocyanin From Strawberries: 1. Solution of pigment was effected from seven and one-half pounds of frozen berries, containing four parts of strawberries to one part of sugar, by extraction with distilled water in proportion of 100 ml. of water for each pound of berries. Heat, not exceeding 48.9°C.(120°F.), was employed. Temperatures above 60.0 to 65.6°C.(140 to 150°F.) must be avoided dur-

ing the process since anthocyanin is readily converted to the anthocyanidin form at these temperatures.

2. The pigment was then separated from other substances in water solution by adding equal quantities of isobutyl alcohol. The anthocyanin was found to be more soluble in isobutyl alcohol than in water, and by thorough mixing in a Waring Blendor for approximately five minutes the greater portion of the pigment could be transferred to the alcoholic phase. Since isobutyl alcohol and water are immiscible, the alcoholic solution could then be easily decanted from the water.

3. The pigment in the crude alcoholic extract was purified by chromatographic separation, by passage of the solution through an adsorption column of alumina. Fisher chromatographic adsorption alumina especially prepared for chromatographic analysis was used in this procedure. A definite blue-purple band appeared at the top of the column. Just above this color band was a faint yellow band, believed to be a flavone.

4. The chromatogram was further developed by washing the column with fresh portions of isobutyl alcohol.

5. The pigment was eluted from the column with distilled water. The small percentage of flavone present on the column was not soluble in the water.

6. The water extract containing the pigment was then centrifuged and filtered to remove any particles of alumina or solid matter that might have come down from the column. The volume of the final pigment extract solution was made up to 750 ml. with distilled water.

Procedure for Isolation of Anthocyanin From Currants: A procedure similar to that employed for strawberries was followed for currants, with certain exceptions. Adsorption of the crude isobutyl alcohol solution on the alumina column disclosed similar bands, except that extracts of currants gave a chromatogram in which the flavone-like material was located just below the anthocyanin band. This material was water-soluble. This necessitated the following modification in the method. Steps One through Three were similar to procedure used with strawberries.

4. The chromatogram was further developed by washing the column with fresh portions of isobutyl alcohol followed by washing with water to remove the small percentage of flavone-like material present on the column.

5. Anthocyanin was eluted from the column with a citric acid solution at pH 2.0. A higher pH value for the eluant did not entirely remove the pigment from the column.

6. The pigment solution was centrifuged, filtered, and made up to volume as in the procedure for strawberry pigment.

Preparation of Samples for Pigment Studies: Samples of the pigment solutions as prepared above were arranged insofar as possible to simulate the conditions provided for study of the fruit juices. Conditions of storage were identical to those previously employed, and observations extended over a period of three months. Eleven lots of six samples each were prepared for observation of the various conditions of experimental treatment and storage. For studies on the effect of the pH of the solution, adjustments of pH values were made with a solution of citric acid (shown Table

5). For studies on the effect of oxygen, one portion of the solution was aerated by mixing in a Waring Blendor for five minutes, and another portion deaerated by bubbling nitrogen gas through the solution for five minutes. To one lot, ascorbic acid was added at the rate of 50 mg. per 100 ml. of solution. Sugar was added to another lot to bring the soluble solids to approximately 15 per cent. The prepared solutions were then filled into small glass vials in 10-ml. portions and sealed, leaving a 10 per cent headspace.

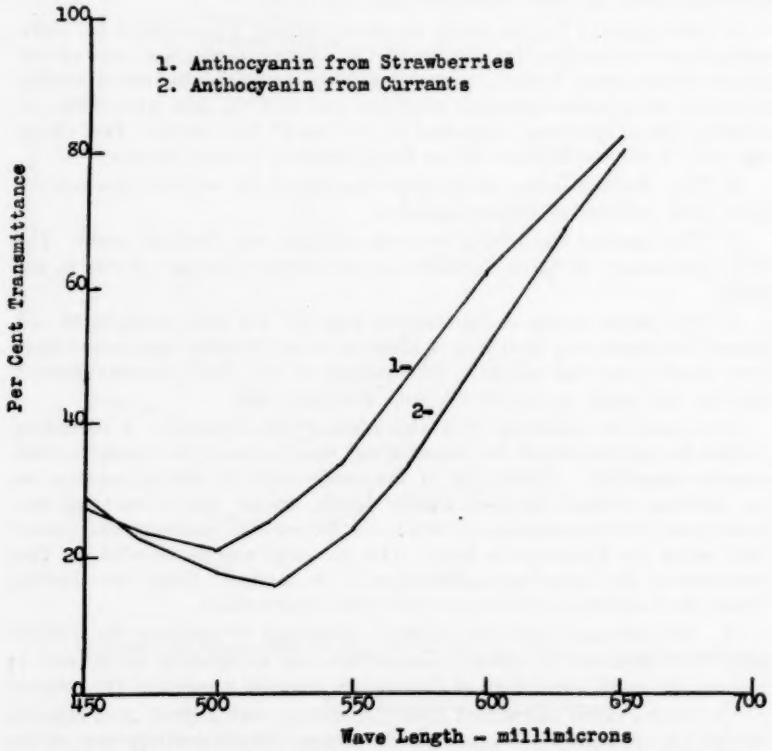

FIG. 3. Light transmittance values on control samples of anthocyanin pigment isolated from strawberries and currants.

Experimental samples of the anthocyanin pigments were removed at intervals of one, two, and three months for color analysis. Analytical procedures were the same as those employed in previous studies. Absorption maxima were determined for the anthocyanin pigment from the respective juices (Fig. 3) and subsequent measurements made at these determined wavelengths. The wavelengths used were 500 mμ. for anthocyanin from strawberry and 520 mμ. for anthocyanin from currant. Results are presented (Tables 4 and 5).

DISCUSSION OF RESULTS

Results obtained in the first part of the investigation indicated that color changes occurring in different fruit juices during storage were not necessarily uniform, but varied with the specific fruit (Tables 1 and 2). In general, all juices showed a change in color during storage. Color changes were most marked in juices which normally possess high color. The stability of the color of blueberry, strawberry, raspberry, and currant juices decreased in descending order. Cherry, grape, and tomato juices were much more stable, and exhibited less color deterioration. Spectrophotometric tests indicated that the percentage transmittance in juices was greatest after storage for three months, and darkening was not observed until after about six months' storage. Results obtained from visual observations also indicated that the juices changed from a bright, clear red to

TABLE 1

Effect of Storage Temperatures on Light Transmittance Characteristics of Fruit Juices

Juice	Period of storage	Temperature of storage			
		—17.8°C. (0°F.)	1.7°C. (35°F.)	21.1 to 26.7°C. (70 to 80°F.)	37.8°C. (100°F.)
		Per cent transmittance [1]			
Blueberry	Control	3	3	3	3
	6 weeks	3	3	3	15
	3 months	5	5	21	68
	6 months	4	4	27	62
Cherry	Control	10	10	10	10
	6 weeks	8	10	11	16
	3 months	10	9	10	15
	6 months	10	9	10	15
Currant	Control	32	32	32	32
	6 weeks	31	32	45	55
	3 months	33	33	55	65
	6 months	30	34	52	51
Grape	Control	13	13	13	13
	6 weeks	13	13	14	19
	3 months	15	14	17	18
	6 months	15	15	19	14
Raspberry	Control	3	3	3	3
	6 weeks	5	3	6	10
	3 months	6	3	21	20
	6 months	5	3	12	16
Strawberry	Control	24	24	24	24
	6 weeks	24	24	28	30
	3 months	25	28	50	55
	6 months	26	30	44	43
Tomato	Control	32	32	32	32
	6 weeks	32	32	32	41
	3 months	34	35	33	35
	6 months	32	34	27	35

[1] Light transmittance values were determined at wavelengths of 500 mμ. for blueberry, cherry, grape, raspberry, and strawberry juices; 475 mμ. for tomato juice; and 450 mμ. for currant juice.

a light, dull red after storage for three months, while darkening was not observed until after six months. This observation, as previously suggested by Pederson *et al.* (1947), reveals two types of changes may have occurred: one, complete destruction of the pigment; and the other, a formation of a new pigment or development of other pigments that are present resulting from reactions between the various constituents present in the juice.

TABLE 2

Effect of Experimental Treatment on Light Transmittance Characteristics of Fruit Juices Stored at 21.1 to 26.7°C.(70 to 80°F.) in the Dark

Juice	Period of storage	Untreated juice	Experimental treatment						
			Stored in daylight	Aerated	Deaerated	Ascorbic[2] acid added	No sugar added	pH [3]	
								Raised	Lowered
		Per cent transmittance [1]						pH 3.5	pH 2.0
Blueberry	Control	3	3	3	3	3	3	3	3
	6 weeks	3	3	10	3	3	2	4	3
	3 months	21	20	50	12	20	9	23	19
	6 months	27	30	38	11	17	8	27	25
								pH 4.25	pH 2.75
Cherry	Control	10	10	10	10	10	10	10	10
	6 weeks	11	10	15	10	10	10	11	9
	3 months	10	11	15	11	13	10	10	18
	6 months	10	10	14	10	13	8	11	9
								pH 4.0	pH 2.75
Currant	Control	32	32	32	32	32	32	32	32
	6 weeks	45	42	55	34	50	43	45	40
	3 months	55	55	65	36	60	52	57	53
	6 months	52	52	62	38	56	52	54	50
								pH 4.0	pH 2.75
Grape	Control	13	13	13	13	13	13	13	13
	6 weeks	14	15	22	14	16	13	16	14
	3 months	17	17	17	15	10	16	17	16
	6 months	19	18	16	14	19	18.5	16	15
								pH 4.0	pH 2.5
Raspberry	Control	3	3	3	3	3	3	5	3
	6 weeks	6	6	8	7	22	6	9	6
	3 months	21	21	22	9	23	19	23	19
	6 months	11	11	16	7	28	14	21	16
								pH 4.25	pH 2.75
Strawberry	Control	24	24	24	24	24	24	24	24
	6 weeks	28	28	30	23	31.5	27	28	23
	3 months	50	49	55	30	62	47	52	52
	6 months	44	40	43	34	52	44	42	40
								pH 4.75	pH 3.75
Tomato	Control	32	32	32	32	32	32	32	32
	6 weeks	32	31	30	32	23	32	32	30
	3 months	33	32	31	33	33	29	33	34
	6 months	27	33	30	32	25	27	36	35

[1] Light transmittance values were determined at wavelengths of 500 mμ. for blueberry, cherry, grape, raspberry, and strawberry juices; 475 mμ. for tomato juice; and 450 mμ. for currant juice.
[2] Ascorbic acid added at the rate of 50 mg. per 100 ml. of juice. [3] Original pH values of the juices were: Blueberry—3.20; cherry—3.50; currant—3.40; grape—3.40; raspberry—3.25; strawberry—3.50; tomato—4.25.

Heat and oxygen content were the most specific accelerating agents responsible for deterioration of color during storage. Increase in temperature of storage or increase in oxygen content resulted in more rapid deterioration of the juices. Deaerated juices stored at 21.1 to 26.7°C.(70 to 80°F.) retained their color and exhibited very little change during storage for six months. Low temperature likewise permitted storage of the juices with little deleterious effect on the color.

Addition of l-ascorbic acid to the juices, except for blueberry and grape juice, accelerated color deterioration, in the form of progressive bleaching. Bleaching was clearly evident in visual examination of stored juices. This effect has been attributed to the reducing action of the ascorbic acid. As previously pointed out by Esselen *et al.* (1946) and other workers in the case of apple juice, addition of ascorbic acid to apple or cranberry juices had a marked effect on lightening color and in retarding darkening during storage. These authors also noted that color and flavor of grape juice were not improved to any great extent by the addition of l-ascorbic acid.

As pointed out by Siemers (1946), ascorbic acid, although effective in retarding discoloration and development of oxidative off-flavors in some fruit products, could not be considered of antioxidant value in retarding color changes in all fruit products. Products such as strawberries and tomatoes, which normally possess a relatively high ascorbic acid content, appeared to derive little benefit from added ascorbic acid.

Beattie *et al.* (1943) observed that an increase in the concentration of ascorbic acid or its isomers to strawberry, currant, or raspberry juices increased the rate of color changes, the rate of change paralleling the loss of ascorbic acid. The same authors also indicated that since the red pigments are readily reducible, and the ascorbic acid readily oxidizable, interaction between the two seems likely.

Comparison of samples stored in light and darkness revealed light had little if any effect on color. Likewise sugar and pH value played only minor roles in regard to stability of color in fruit juices. Addition of sufficient concentrations of sugar (one part of sugar to one, two, or three parts of strawberries) to strawberry fountain syrup preserved the bright-red color when products were stored at from 21.1 to 26.7°C.(70 to 80°F.) for periods of six months (Table 3). Addition of citric acid to packs containing lower concentrations of sugar (one part of sugar to four and five parts strawberry) did have some preservative effect on the color during similar conditions of storage.

Results obtained in the second phase of the investigation showed that the stability of the anthocyanin pigments isolated from strawberries and currants were subject to some variation (Tables 4 and 5). Increased storage temperatures and the presence of oxygen accelerated color deterioration with both pigments, while a low storage temperature generally preserved the color. Deaerated samples of the isolated pigments stored at from 21.1 to 26.7°C.(70 to 80°F.) retained their color and exhibited very little change during storage for three months. Added l-ascorbic acid had a bleaching effect on the color of the pigment from strawberries and greater bleaching action on the pigment from currants, ultimately resulting in nearly complete decolorization of the pigment solution. Light, on the other

hand, caused rapid bleaching of the pigment from strawberries, but had only a moderate effect on the pigment from currants.

Of interest was the fact that deteriorative effects resulting from exposure to light occurred in solutions of isolated pigments but not in the juices of these same fruits. These observations suggest a number of possible explanations. A change in chemical structure of the pigments may have occurred during the process of isolation which rendered the pigment more susceptible to photolytic decomposition. Also probable is the possibility that certain constituents present in the juice may have exerted protective effects on the pigments which would be lacking in solution of isolated pigment. Another possibility is the formation or development of new pigments resulting from chemical reactions between various ingredients of the fruit. Reactions of this latter type may be the cause of alternate bleaching and darkening observed with certain of the juices.

TABLE 3

Effect of Different Concentrations of Sugar and Citric Acid on Light Transmittance Characteristics of Strawberry Fountain Syrup Stored at 21.1 to 26.7°C. (70 to 80°F.) in the Dark

Sample[1]	Control	Period of storage		
		6 weeks	3 months	6 months
		Per cent transmittance at 450 mμ.		
A	19	29	39	24
A1	19	25	27	16
A2	19	26	31	22
B	19	27	34	24
B1	19	23	25	16
B2	19	25	28	18
C	19	19	24	20
C1	19	17	23	19
C2	19	19	19	20
D	19	22	20	24
D1	19	18	24	19
D2	19	20	22	22
E	19	21	22	20
E1	19	19	19	19
E2	19	19	19	19

[1]A—5 parts strawberries to one part sugar; B—4 parts strawberries to one part sugar; C—3 parts strawberries to one part sugar; D—2 parts strawberries to one part sugar; E—1 part strawberries to one part sugar; 1—0.5 per cent by weight citric acid added; 2—0.25 per cent by weight citric acid added.

Decreasing pH values appeared to intensify and protect the color during storage, whereas increasing pH values diminished the intensity of color and increased deteriorative effects during storage.

The anthocyanin pigment solution isolated from strawberries developed a faint precipitate after storage for a period of one week. No such precipitation occurred in the anthocyanin pigment solution isolated from the currants. It was also noted that the degree of precipitation increased progressively during storage for periods up to six months. This precipitation

may have occurred as a result of acid hydrolysis which converted the anthocyanin (glucoside) to the water-soluble anthocyanidin. Precipitation of the pigment from strawberries and not from currants was attributed possibly to a lower stability of the strawberry anthocyanin.

TABLE 4

Effect of Storage Temperatures on Light Transmittance Characteristics of the Anthocyanin Pigments Isolated From Strawberries and Currants

Pigment	Period of storage	Temperature of storage			
		—17.8°C. (0°F.)	1.7°C. (35°F.)	21.1 to 26.7°C. (70 to 80°F.)	37.8°C. (100°F.)
		Per cent transmittance [1]			
Anthocyanin from strawberry	Control	20.0	20.0	20.0	20.0
	1 month	22.5	22.5	28.5	36.5
	2 months	23.0	21.0	26.0	39.0
	3 months	23.0	22.0	27.0	50.0
Anthocyanin from currant	Control	16.5	16.5	16.5	16.5
	1 month	17.0	17.0	19.5	24.0
	2 months	16.5	17.0	20.0	31.0
	3 months	16.5	17.0	24.0	56.0

[1] Light transmittance values were determined at wavelengths of 500 mμ. and 520 mμ. for strawberry and currant pigments, respectively.

TABLE 5

Effect of Experimental Treatment on Light Transmittance Characteristics of the Anthocyanin Pigments Isolated From Strawberries and Currants Stored at 21.1 to 26.7°C.(70 to 80°F.) in the Dark

Pigment	Period of storage	Experimental treatment							
		Untreated pigment	Stored in daylight	Aerated	Deaerated	Ascorbic[2] acid added	Sugar added	pH [3] Raised	pH [3] Lowered
		Per cent transmittance [1]						pH 4.5	pH 2.5
Anthocyanin from strawberry	Control	20.0	20.0	20.0	20.0	20.0	20.0	40.0	5.5
	1 month	28.5	64.5	36.0	23.0	29.0	25.5	49.0	5.5
	2 months	26.0	65.5	40.0	24.0	34.0	27.0	52.0	6.0
	3 months	27.0	72.0	44.0	25.0	34.0	25.5	55.0	5.5
								pH 3.0	pH 2.0
Anthocyanin from currant	Control	16.5	16.5	16.5	16.5	16.5	16.5	25.0	18.0
	1 month	19.5	24.5	24.0	18.0	74.0	21.5	28.0	20.0
	2 months	20.0	28.0	30.0	19.0	74.0	21.5	28.0	20.0
	3 months	24.0	37.0	38.0	21.0	74.0	.....	34.0	22.0

[1] Light transmittance values were determined at wave lengths of 500 mμ. and 520 mμ. for pigments from strawberry and currant, respectively. [2] Ascorbic acid added at the rate of 50 mg. per 100 ml. of sample. [3] Original pH values of the pigments were strawberry—3.5, currant—2.5.

SUMMARY AND CONCLUSIONS

Studies were made on the effect of length and temperature of storage, and the relationship of oxygen, light, sugar, pH, and ascorbic acid to deteriorative changes in the color of seven representative fruit juices and strawberry fountain syrup. Similar studies were made on solutions of purified anthocyanin pigments isolated from strawberries and currants to observe whether deteriorative changes in juices of these fruits were directly associated with changes in these pigments.

Temperature of storage and oxygen content were the most specific agents responsible for deterioration of color during storage of both juices and isolated pigments.

Exposure to light caused little deterioration of color in juices, but exerted a bleaching effect on the isolated anthocyanin pigments.

Adjustment of pH values had little if any effect on deterioration of color in fruit juices during storage, but some effect was observed in solutions of isolated pigments. Lower pH values exerted a protective action on pigment solutions.

Sugar had little effect on stability of color in either juices or pigment solutions.

When used as an antioxidant, l-ascorbic acid exerted no marked protective properties in respect to color stability. With all the juices except blueberry and grape juice, progressive bleaching resulted from addition of ascorbic acid in concentration of 50 mg. per 100 ml. of juice. Addition of similar concentrations of solutions of strawberry anthocyanin resulted in virtually complete decolorization of the pigment.

Increasing sugar and/or citric acid concentration enhanced the stability of the color of strawberry fountain syrup during storage.

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THE pH OF FRUIT JUICES

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During the past five years, acidities of fruits brought into the laboratory for various experimental and cookery purposes have been recorded. The range of data for each fruit is presented herein.

EXPERIMENTAL PROCEDURE

Sampling of Fruit: The fresh fruit samples represented whole, sound, mature pieces of fruit selected from local markets. Under retail conditions of marketing, no dependable information was available as to the variables which contribute to total acidity of fruit—such as conditions of harvesting, shipping, storage temperatures, and varieties.

The fresh fruit data recorded represent the acidity of the fruits with which cookery processes are concerned. Some of the fruits were purchased frozen and in some cases were frozen to expedite the extraction of juice. Preliminary tests on pH of fruits before and after freezing showed no differences measurable under the conditions of the tests. Every effort was made to select fully ripe fruit at the peak of its flavor excellence; nearness to the area of production made this possible.

DATA

Acidity of the prepared juices was measured by glass electrode at 20°C. (68°F.). Results are given (Table 1).

DISCUSSION OF DATA

The overall range of pH for the 27 fruits tested is roughly two to five, omitting watermelon. Three-fourths of the 382 samples tested fell within the range three to four, if cactus, certain blue grapes, oranges, persimmons, and watermelon are not considered. Values reported in the three references included in the table are, as a whole, in agreement with the central tendency of the "new data" as expressed in the second column of the data.

It is the pH rather than titratable acidity of fruits which is of particular significance in those cookery processes that involve precipitation of colloid sols, as in cheese- and jelly-making, carbohydrate hydrolysis, in sugar and starch cookery, and the liberation of CO_2 from carbonates in leavening.

Seven organic acids and their salts are recognized as characteristic of fruits: four of the seven are the common polybasic acids — citric, malic, tartaric, and oxalic; a fifth polybasic acid — succinic — can also be identified in certain fruits; benzoic acid occurs in cranberries and certain plums; acetic acid has been reported in figs by Nelson (1928).

TABLE 1

Characteristic Acidity of Fruit Purchased for Cooking Purposes (Expressed as pH)

Name of fruit	New data			Data from references		
	No. of samples	Ranges		3 [1]	4 [2]	5 [3]
		Overall	3/4 of samples			
Apples	30	3.00-5.00	3.2-3.5	.....	2.9-3.3	3.00-5.49
Apricots	12	3.40-4.00	3.6-3.8	3.3	.....	.....
Bananas	0	.....	.....	4.7	.....	.....
Blackberries	24	2.80-3.95	3.3-3.6	3.3	3.3-3.6	.....
Blueberries	6	3.00-3.85	3.6-3.8	.....	.....	.....
Cactus tuna	7	4.10-4.50	4.2-4.4	.....	.....	.....
Cherries	....	.....	.....	.....	3.2-4.1	.....
Cherries (sour)	18	2.50-3.70	3.3-3.4	.....	.....	.....
Cherries (sweet)	12	3.20-3.95	3.4-3.7	3.8	.....	.....
Cranberries	12	2.40-2.95	2.7-2.9	.....	.....	.....
Currants (red)	6	2.80-3.50	3.0-3.1	.....	.....	.....
Figs	0	.....	.....	4.7	.....	.....
Grapes (blue)	12	2.50-4.50	3.1-3.4	.....	3.5-4.5	2.57-3.28
Grapefruit	30	3.00-3.75	3.2-3.4	.....	3.0-3.3	.....
Gooseberries (green)	8	2.60-3.80	2.7-3.1	3.0	2.8-3.1	.....
Lemons	12	2.20-3.50	2.7-3.1	2.2	2.2-2.4	.....
Limes	12	1.70-2.95	2.0-2.7	2.0	1.8-2.0	.....
Oranges	18	3.55-4.90	3.5-3.9	3.4-4.1	3.0-4.0	.....
Peaches	20	3.20-3.90	3.5-3.8	3.5	3.4-3.6	.....
Pears	12	3.20-3.95	3.7-3.8	3.9	3.6-4.0	.....
Persimmons	9	4.30-4.95	4.4-4.8	.....	.....	.....
Pineapples	6	3.20-3.62	3.3-3.5	.....	.....	.....
Plums (cultivated)	24	2.80-3.40	3.1-3.2	.....	.....	.....
Pomegranates	12	3.00-3.45	3.1-3.4	.....	.....	.....
Prunes	20	2.90-3.70	3.2-3.4	3.1	.....	.....
Raspberries	0	.....	.....	3.6	3.2-3.7	.....
Raspberries (red)	16	3.00-4.00	3.1-3.6	.....	.....	.....
Raspberries (black)	4	3.20-3.90	3.3-3.7	.....	.....	.....
Rhubarb	0	.....	.....	3.2	.....	.....
Strawberries	24	3.10-4.00	3.2-3.5	3.4	3.1-3.5	.....
Tangerines	12	3.80-4.40	3.9-4.2	.....	.....	.....
Watermelon	4	6.00-6.40	6.0-6.3	.....	.....	.....

[1] Reference 1; [2] Reference 5; [3] Reference 2.

Total acidity measurements variously reported in textbooks are from 0.1 to 11 per cent citric. Charley (1947) measured the acidity of 19 of the more common fruits and found the acidity of apples to be from 0.1 to 1.2 per cent and that of lemons four to eight per cent each citric. Charley did not include limes, a fruit commonly more acid than lemons. Few workers have reported the stage of ripeness of the fruit either as picked or as tested; often these are variables more significant than the variety names. These niceties of description of the fruit tested are not possible, except when work is done with fruit harvested from known nurseries, as a cooperative project with horticulture.

The pH value of a sample of fruit is, of course, the resultant of the buffer equilibrium of the various acids and salts involved in titrations; yet there is no consistent, positive correlation between titratable acidity and pH when measurements represent random samplings of fruit, such

as consumers must use. When comparisons of these values represent fruits picked from the same plants where heredity factors are constant, the correlations are, of course, more definite, but not always high, inasmuch as environmental conditions vary from year to year, as well as from early to late season.

SUMMARY

A record is made of the pH of 382 samples of 27 kinds of fruit purchased for experimental and cookery purposes in connection with work done at the University of Texas. The overall range of pH is two to five; the most frequent range is between three and four. The importance of pH rather than total acid of fruits in cookery processes is emphasized.

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USE OF REPLICATIONS IN DEEP-FAT FRYING EXPERIMENTS[1]

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The design of experiments for deep-fat frying has received little attention, though this method is popular for studying the behavior of fats. The necessity for replicate determinations is an accepted fact in most fields of research, yet the use of replications in frying tests has been reported by only one group of workers, King, Loughlin, Riemenschneider, and Ellis (1936).

In the study herewith reported data are presented which demonstrate the need for replication in deep-fat frying tests.

"Large quantities of oils and fats are used in the commercial frying of such products as doughnuts, potato chips, nuts, etc., as well as in the preparation of a variety of fried foods in restaurants and hotels"—Bailey (1945). The suitability of a fat for deep-frying is frequently studied by means of frying tests. Criteria which help determine its value for this purpose include fat absorption by the food during frying, chemical and physical changes in the fat during the course of the cooking operation, and characteristics of food cooked in the fat. The frying method has been used not only to test the stability of fats for this cooking process as exemplified by the work of Morgan and Cozens (1919), Lowe, Nelson, and Buchanan (1940), and others but also to determine the effect of different doughnut ingredients on fat absorption, as has been done by Denton, Wengel, and Pritchett (1920), Thiessen (1939), and others. The frying test has also been used for the purpose of gaining information of theoretical value concerning the behavior of fats. The study of Porter, Michaelis, and Shay (1932), which dealt with changes in fats during frying, was conducted primarily to gain information as to the substances formed which impart objectionable flavors to foods.

The highly complex nature of most foods suggests that, in the handling of such materials, there are almost certain to be variations resulting from factors that cannot be controlled. Such variations have been noted by many workers in the field of foods. Lowe *et al.* (1940), in their studies of deep-frying, emphasize the fact that no two mixes of dough are alike. They state, "The technic in handling the dough became highly specialized, and although no two batches of dough were exact duplicates, constant working with one type of dough tends to minimize these differences in mixes." And again, "The two previous units of the study had shown

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the importance of the individual mix, in that one mix was not a duplicate of another mix, just as no two persons' fingerprints are alike." King, Loughlin, Riemenschneider, and Ellis (1936) compared the relative value for frying of several lards and other fats, and found by means of chemical tests that all deteriorated during use. When the frying tests were repeated, using fresh portions of each fat, the extent of chemical change was found to differ from that observed in the first tests. Bailey (1945) observed: "The unusually extensive tests of Lantz and Carlin reveal considerable differences among five representative lots of vegetable shortenings, but it is uncertain to what extent these are due to unavoidable variables in the frying operation."

In order to distinguish between the variations due to known factors under investigation, and those due to unknown and hence uncontrollable factors, statistical methods must be used in the analysis of the data. Hence, the experiment must include replications. This is a bygone conclusion in many fields of research. "The purpose of replication is two-fold. On the one hand it is used to obtain a sufficient degree of accuracy in the results, and on the other hand it provides a means for the calculation of experimental error." American Association of Cereal Chemists, Cereal Laboratory Methods (1941).

The customary procedure of doing but one series of fryings in each fat has been followed, partly because of the time consumed by a frying test, and frequently because the amount of fat available was too small to allow conveniently for replications. It has been assumed that, if conducted according to a controlled procedure, results of the one test would be representative for each fat.

The purpose of the experiment herewith reported was to test the validity of this assumption.

EXPERIMENTAL PROCEDURE

A comparison was made of the behavior during their use for frying of a number of portions of the same fat. Two fats were studied separately according to the following plan: A quantity of fat was melted, and equal amounts weighed into each of four aluminum kettles of the same size and shape. Twenty-four lots of six doughnuts each were fried in each portion of fat. The order of use of the portions was randomized for each time of frying. This is equivalent to a randomized-block experiment, with the 24 times of frying being the 24 blocks.

Doughnuts were prepared by an experienced operator having a highly standardized technique. All steps in the preparation of the doughnuts were timed by stopwatch. Room and ingredient temperatures were maintained at 25°C.(77°F.), and the temperature of the different portions of fat during frying was maintained at 185°C.(365°F.). Samples of the fat were taken for chemical analysis after frying each 12 doughnuts; samples of the fried food were also taken from each dozen. No fresh fat was added to the kettles during the frying process.

The following observations were made:

Absorption of fat (kettle weight before and after frying each lot of six doughnuts).

Moisture loss from doughnuts during frying (fat absorbed minus gain in weight of doughnuts).

Chemical changes in the fats during use for frying.

Chemical changes in the fat extracted from the doughnuts.

RESULTS AND DISCUSSION

The analysis of variance, Snedecor (1940), was used in testing the significance of the differences among the four portions of the same fat. The 24 times that each portion was used for frying were used as replications in making this comparison. The variation due to time was removed from that due to experimental error. The number of degrees of freedom for the portions, time, and error were three, 23, and 69, respectively. Results are shown (Tables 1 and 2).

Moisture Loss: Moisture loss from the doughnuts during cooking is a measure of the uniformity of time of cooking and temperature maintained during the frying operation. The analysis of variance of moisture loss by doughnuts during frying in Lard I is shown (Table 1).

TABLE 1

Analysis of Variance of Moisture Loss

Source of variation	Degree of freedom	Sum of squares	Mean squares	F
Portion of fat	3	00.7114	0.2371	0.87
Time (replication)	23	42.2402	1.8365	.....
Error	69	18.8247	0.2728	.....
Total	95	61.7763	.....	.....

There was no significant difference among the four portions of fat in loss of moisture from the doughnuts during cooking. This indicates that cooking time and temperature did not vary significantly from portion to portion. This was also true for Lard VI.

Fat absorption and chemical changes in each portion of fat were analyzed by the same statistical method. Details of the analyses are omitted and only the variance-ratios, F, with three and 69 degrees of freedom, are shown (Table 2).

Fat Absorption: When calculated on the basis of total fat absorbed, there was no significant difference among the four portions in fat absorption by the doughnuts during cooking. This was true for both Lard I and Lard VI. The raw doughnuts varied somewhat in weight. When fat absorption was calculated on the basis of grams per 100 grams of raw doughnut, the analysis of variance showed no significant difference among the portions for Lard VI, but a significant difference for Lard I.

Whether fat absorption should be reported on the basis of total fat absorbed, or on the basis of per cent absorbed is open to question. Lowe *et al.* (1940) state, "The grams of fat absorbed rather than the percentage of fat absorbed were used for the statistical calculations. Since the dough was always rolled the same thickness and the same cutter used, the surface area for absorption of fat by a lot of six doughnuts or a mix was approximately the same. . . . it was thought this was a fairer basis for comparisons

of fat absorption than the percentage of fat absorbed as calculated on either the cooked or uncooked weight."

Iodine Numbers: For both Lard I and Lard VI, the iodine numbers showed highly significant differences among the four portions of the same fat. This was true both for fat taken from the kettles and for fat extracted from the doughnuts.

TABLE 2

Test of Significance of Differences Among Four Portions of the Same Fat

Characteristic	F with 3 and 69 d.f.[1]	
	Lard I	Lard VI
Moisture loss from doughnuts during cooking	0.87	1.13
Fat absorption by doughnuts	2.84	1.14
Fat absorption by doughnuts on basis of grams' absorption per 100 grams raw weight	4.10	0.53
Iodine numbers		
Kettle fat	9.13	6.12
Extracted fat	5.07	10.89
Free fatty acids		
Kettle fat	1.40	3.83
Extracted fat	1.41	5.88
Peroxide numbers		
Kettle fat	2.09	3.19
Extracted fat	3.49	0.18

[1] The five per cent and one per cent points are 2.74 and 4.08, respectively.

Free Fatty Acids: Free fatty acids showed no significant differences among the four portions of Lard I. This was true both for fat taken from the kettles and for fat extracted from the doughnuts. For Lard VI, differences were significant at the five per cent level for fat taken from the kettles, and at the one per cent level for fat extracted from the doughnuts.

Peroxide Numbers: Peroxide numbers for the four portions of fat showed no significant difference for Lard I, but differences among portions were significant at the five per cent level for Lard VI. Differences in peroxide numbers for fat extracted from the doughnuts were significant at the five per cent level for Lard I and not significant for Lard VI.

CONCLUSION

The experiment herewith reported has shown that different portions of the same fat do not behave alike in a deep-fat frying test. Since one portion is not representative of the whole, repeated observations on but one portion cannot be considered replications in comparing different fats. The experiment must be replicated by using more than one portion of each of the fats being studied. In testing the significance of differences among a number of fats, the correct experimental error will be the variation among different portions of the same fat. For an experiment of a fixed size, which might be limited by the quantity of material available or other factor, more than one portion of fat should be used, though this be at the expense of the number of times of frying.

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THE OXIDASE OF CAULIFLOWER

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Freezing preservation of vegetables, including cauliflower, has greatly increased in the past few years, and with this increase the prevention of deterioration in flavor, odor, and color during freezing storage has assumed greater importance. As indicated by Joslyn (1946) and others, there is good evidence to indicate enzymes are directly involved in formation of off-flavors and odors, and possibly, darkening of the color, due to the production of intermediates when tissue cells have been injured by freezing.

Of the enzymes naturally occurring in vegetables, the oxidases have been found to be the most useful indicators of the tendency for various frozen vegetables and fruits to deteriorate in freezing storage.

Blanching in steam or hot water is the method used industrially to inactivate enzymes or to minimize their activity in frozen vegetables. The relative activity of the naturally occurring oxidase, particularly that of peroxidase, is usually the criterion of adequate blanching.

Balls (1942, 1943) in research in connection with dehydration of vegetables, found it is difficult, in commerical practice, to inactivate completely the oxidizing enzymes of some vegetables, and that regeneration of the heat-inactivated enzymes can occur, particularly in cabbage. Before our research was undertaken there were no published data to show that significant regeneration of heat-inactivated, oxidizing enzymes occurs in frozen vegetables.

Classification and general properties of the oxidizing enzymes of plant and animal tissues are well presented in the recent book of Sumner and Somers (1947), hence we shall not review such information in detail. It will suffice to state that oxidases and catalase are classified into: (1) the iron-containing enzymes: peroxidase, cytochrome oxidase, and catalase; and (2) the copper-containing oxidases: phenol oxidases (monophenol oxidase, tyrosinase, polyphenol oxidase, and lactase) and ascorbic acid oxidase. As shown later in this paper, cauliflower does not contain phenolases. Cytochrome oxidase was omitted from our studies since Lipton, Arnold, and Berger (1939) have shown it is destroyed by freezing and thawing. Catalase was also omitted by us, because it is not a true oxidizing enzyme.

Johnson and Zilva (1937) demonstrated the presence of peroxidase in cauliflower.

Tauber, Kleiner, and Mishkind (1935) appear to be the first to report the presence of ascorbic acid oxidase in plant tissues; in their case, in Hubbard squash *(cucurbita maxima)*. They reported more fully in a second paper in 1936. Later Hopkins and Morgan (1936) found this enzyme in cauliflower, and their work was confirmed by Kertesz (1938). Johnson and Zilva (1937) concluded that the ascorbic acid oxidases of

cauliflower and other vegetables are identical, and Zilva (1936) found ascorbase is not entirely specific, since it catalyzes the oxidation also of d-gluco ascorbic acid which has no anti-scorbutic properties.

Kertesz, Dearborn, and Mack (1936) state that ascorbic acid oxidase is inactivated by heat at about the same rate as catalase, and is very much less resistant to heat than is peroxidase. An interesting observation is that of Reder (1946) — that boiled cauliflower extract inhibits oxidation of ascorbic acid as much as 70 per cent. He suggests this inhibition may be due to –SS– or –SH– groups. Crook and Hopkins (1938) had previously observed that cauliflower juice catalyzed the reduction of dehydroascorbic acid.

EXPERIMENTAL WORK

This investigation was concerned chiefly with the properties of the peroxidase and the ascorbic acid oxidase of cauliflower, although some attention was given to the natural oxidase substrate of cauliflower, other than ascorbic acid, and to determining whether or not certain dehydrogenases and phenolase were present.

I. *Peroxidase:* Attention will be given, first, to the preparation of a purified solution of this enzyme and to its properties, including regeneration after heat inactivation.

Preparation of Peroxidase Solution: Fresh cauliflower was ground in a kitchen-size food grinder, then mixed for three minutes in a Waring Blendor, with twice its weight of acetone. The mixture was filtered, the solid residue "blended" again with the volume of acetone mentioned above, and filtered; the residue was treated again with acetone and filtered, and the residue on the filter paper air-dried to a white powder. To this powder, in a mortar, was added acetate buffer of pH 5.38 of a volume in c.c. equal to the weight of the fresh cauliflower. The mixture was ground three minutes with white quartz sand and filtered after 10 minutes' steeping. Usually a brilliantly clear water-white and very active (in respect to peroxidase) preparation was obtained. In cases where the filtrate was not brilliantly clear, it became so on freezing for about 24 hours, thawing, and filtering.

This method of preparation eliminates catalase, which would interfere with peroxidase measurement by destroying H_2O_2, and removes phenolic substances, tannins, and probably other organic substances that might interfere with the peroxidase activity.

In order to reduce enzyme denaturation to a minimum, very cold acetone, —10°C.(14°F.), was used. A buffer of pH 5.38 was employed, since this pH was found to be the optimum for oxidation of guaiacol by cauliflower peroxidase.

Qualitative Tests for Presence of Phenolase, Peroxidase, and Catalase: First tests were made upon the scarified tissue slices of raw cauliflower. Oxidizable substitutes used were ten monohydroxy compounds, including alpha napthal, p-cresol, and o-cresol; six di-hydroxy indicators, including catechol, hydroquinone adrenaline hydrochloride, and resorcinol; five trihydroxy compounds, including pyrogallol, gallic acid, and phloroglucinol; one penta-hydroxy compound, quercetin; five mono-amino compounds, including o-, m-, and p-toluidine; four di-amino compounds, including

benzidine and o-, m-, and p-phenylene-diamine; six mono-hydroxy mono-amino compounds, including l-tyrosine, o-, m-, and p-aminophenol, and 5-amino salicylic acid hydrochloride; and one mono-hydroxy di-amino compound, 2-amino 6-nitrophenol. Three to four drops of each of the dilute (0.5 per cent) solutions of the various substrates were placed on the cut surfaces without adding H_2O_2. Reactions with all these indicators were negative, indicating the absence of phenolase.

When dilute H_2O_2 was added with these indicators color reactions were positive, except with l-tyrosine and adrenaline hydrochloride, showing the presence of an active peroxidase.

With H_2O_2 alone, O_2 gas evolved vigorously, proving catalase was present.

Similar results were obtained with the purified enzyme extract, except that catalase was absent.

Measurement of Peroxidase Activity: The well-known colorimetric method with guaiacol, as used by Hussein and Cruess (1940) and Ponting and Joslyn (1948), was modified to improve its reliability and accuracy. As modified, it is as follows: To an Evelyn colorimeter tube containing 10 ml. of acetate buffer of pH 5.38 is added one ml. of 0.5 per cent guaiacol in 50 per cent ethyl alcohol and one ml. of .05 N H_2O_2. Then one ml. of the enzyme preparation is added and mixed with the solution in the tube. The tube is placed in an Evelyn colorimeter and readings taken at 15-second intervals, with a 420-μ filter inserted. Previously the galvanometer is set at 100, using a tube containing the reagents mentioned above, but not the enzyme preparation.

If G is the per cent of light transmitted, the corrected values for 2-log G are obtained from the colorimeter chart and plotted against time. In the activity curve there is an initial short lag period after which the values for 2-log G give a straight line for several minutes, indicating a first-order reaction. The activity of the enzyme preparation is given by the slope of the straight line and is expressed in 2-log G units per minute. With most of our preparations the activity could be determined in four minutes; with weak preparations, in about six minutes.

The method is sensitive, and results are reproducible within five per cent. Fairly accurate measurements can be made with solutions too weak to show appreciable activity by the titrimetric method of Balls and Hale (1934). The colorimetric method can be standardized against the method of Balls and Hale, which has the advantage of giving results in moles or milligrams of H_2O_2 used.

We found that the activity obtained by plotting 2-log G against time over a four- to six-minute period is more reliable than arresting the reaction after a definite interval by addition of HAc or KCN, as they cause some bleaching of the indicator color, and there is also the danger of stopping the reaction, when it is not in the first-order reaction range.

We used all reagents and made all readings at room temperature, about 24°C.(75°F.), which was sufficiently constant during a given series of readings in a single experiment. The buffer solution at pH 5.38 gives a brilliantly clear solution of the oxidized guaiacol (tetra-guaiacoquinone), free of disturbing haze often encountered at some pH values.

The enzyme preparations were tested for presence of catalase by the method of Euler and Josephson (1927), as modified by Sumner and Somers (1947). Catalase was uniformly absent.

Location of Peroxidase in Cauliflower: Thin cross-sections of cauliflower stem were immersed in dilute alcoholic solutions of pyrogallol, guaiacol, catechol, and p-phenylene diamine for a short time. The indicator solutions were then diluted and a few drops of two per cent H_2O_2 added. The p-phenylene diamine gave the most pronounced and sharpest color.

The most rapid and intense development of color occurred in the vascular layer, especially in the cells surrounding the fibro-vascular bundles. The epidermal layer beneath the skin was next in intensity. The parenchyma tissue showed the slowest development of color, which was uniform throughout. These observations agree well with those of previous investigators working with other plants. The flower buds (''curds'') also gave intense peroxidase color reactions with the indicators used.

Effect of Enzyme Concentration: The purified enzyme preparation was diluted five, 10, 15, 20, and 50 to one with a buffer solution, and peroxidase activity determined, as previously described. Up to a concentration of 15 to one the activity was directly proportional to the dilution, while at greater dilutions the activity decreased at a faster rate than the dilution increased. This is possibly due to the fact that the ratio H_2O_2 to enzyme increased with increasing dilution and the resulting excess of H_2O_2 per unit of enzyme caused some inhibition. By the same token, the guaiacol increase per unit of enzyme insured the necessary excess of substrate. Possibly also denaturation of the enzyme may occur at greater dilution. In our experiments on other points we used dilutions of less than 15 to one.

Sumner and Somers (1947) point out that in experiments on effect of dilution of the enzyme solution on its activity, the concentration of H_2O_2 must be very low and an excess of substrate must be available.

Effect of Temperature on Activity: Several Evelyn colorimeter tubes containing 10 ml. of citric acid-phosphate buffer of pH 5.57, 0.5 ml. of 0.5 per cent guaiacol, and 0.5 ml. of 0.05 N H_2O_2 were held at various temperatures until they attained temperature equilibrium. Then one ml. of enzyme solution was added and the reaction allowed to proceed for three minutes. It was then stopped by addition of KCN and the color read on an Evelyn colorimeter. Since in most cases the tubes were at temperatures different from that of the colorimeter, it was necessary in this experiment to arrest the reaction, rather than take a series of readings while the reaction was in progress, as was done in other experiments.

The data are shown graphically in the accompanying diagram (Fig. 1). It will be seen that the optimum temperature in these tests was at about 30 to 34°C.(86 to 93°F.). At 40°C.(104°F.) definite inhibition was apparent. There was appreciable activity at 0°C.(32°F.).

Inactivation by Heat: It is well known that if peroxidase is not inactivated by blanching vegetables in steam or hot water, undesirable odors and flavors are likely to develop during subsequent freezing storage; although this enzyme may not be chiefly responsible, it acts as an index for such deterioration.

Joslyn (1946) states that over three minutes at 100°C.(212°F.) or over nine minutes at 82°C.(180°F.) are required to inactivate the peroxidase of cauliflower in the tissues; and over two minutes at 100°C. or over six minutes at 82°C., in the juice.

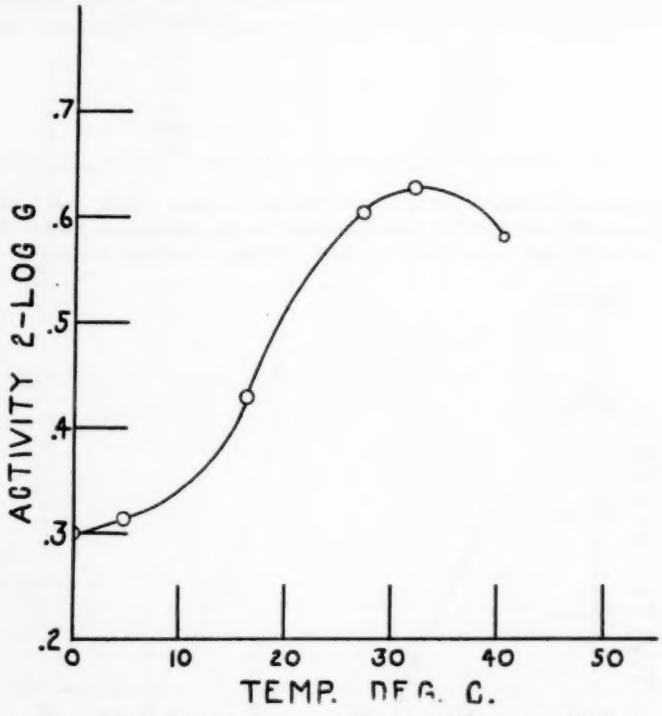

FIG. 1. Effect of temperature on activity of cauliflower peroxidase.

In our determination of the inactivation temperature, small volumes (five c.c.) of the purified enzyme solution were heated for five minutes at temperatures ranging from 55 to 100°C.(131 to 212° F.), after which the tubes were cooled at once in an ice and water mixture. The activity of each lot was then determined at room temperature. Results are shown in the accompanying graph (Fig. 2). The enzyme was inactivated at about 90°C.(194°F.) at pH 5.38 in five minutes. Its activity was materially decreased by five minutes at 55°C.(131°F.).

Effect of Hydrogen Peroxide Concentration: Using an enzyme preparation of pH 5.38 and the colorimetric technique previously outlined, it was found that cauliflower peroxidase, like that of other plants, is sensitive to H_2O_2. It will be noted (Table 1) that in our experiments the optimum H_2O_2 concentration appeared to be in the range of 5×10^{-3} M

to 1×10^{-3} M. Also, at very low concentrations the rate is retarded by insufficient H_2O_2 to maintain maximum activity.

TABLE 1

Effect of H_2O_2 Concentration on Activity of Peroxidase

H_2O_2 molar concentration	Activity (2-log G)
1×10^{-2}	.312
5×10^{-3}	.541
2.5×10^{-3}	.611
1×10^{-3}	.541
5×10^{-4}	.300
1×10^{-4}	.072

Effect of Hydrogen Ion Concentration: Using Clark and Lub's biphthalate-HCl acid buffer for pH four to six, phosphate buffer for pH six to eight, and boric acid (in KCl solution)—sodium hydroxide buffer

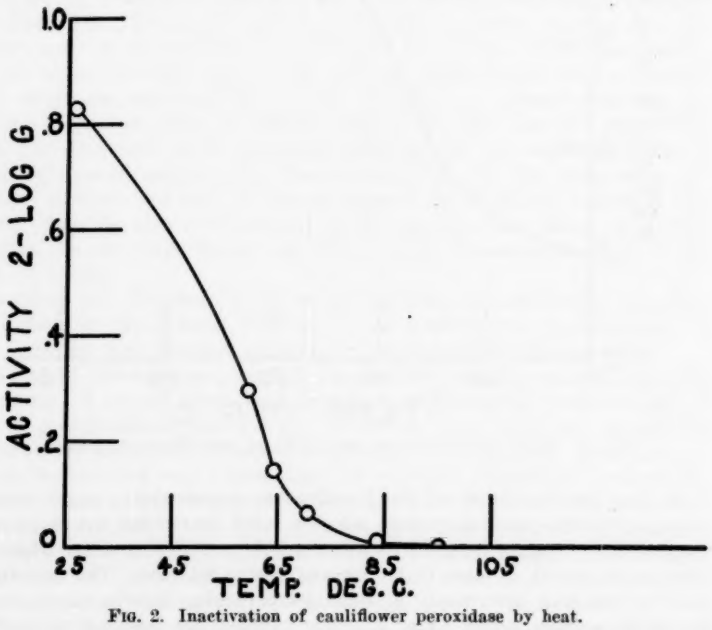

FIG. 2. Inactivation of cauliflower peroxidase by heat.

for pH eight to 10, the activity of the purified peroxidase solution was determined at various pH values. The enzyme was allowed to react with the various buffers for 30 minutes before the guaiacol and H_2O_2 were added and the activity determined. Data are presented (Fig. 3).

The optimum pH range with guaiacol as the substrate was approximately 5.2 to 5.6, which agrees reasonably well with the observations of other investigators on peroxidase from other sources, such as Cruess and

Fong (1929) with apricot peroxidase, Bansi and Ucho (1926) with horseradish peroxidase, Jimenez (1947) with guava peroxidase, and others.

Effect of Several Halides: Sodium fluoride caused complete inhibition of the peroxidase at 3.5×10^{-2} M concentration and 40 per cent inhibition at 0.001 M. These results agree well with those of Hussein and Cruess (1940) for grape peroxidase and Jiminez (1947) for guava peroxidase. Sodium chloride caused about 44 per cent inhibition at 7.5×10^{-5} M,

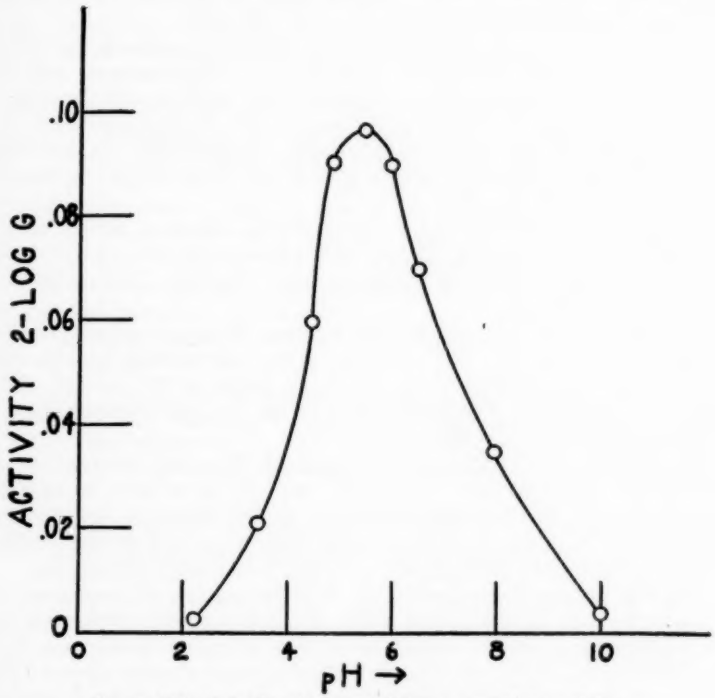

FIG. 3. Effect of pH value on cauliflower peroxidase activity.

and NaBr 50 per cent inhibition at 5×10^{-5} M. At concentrations above these the activity increased; eventually attaining almost that of the untreated check. Since in the presence of acid H_2O_2 liberates free iodine from NaI, the effect of the iodide could not be followed.

Effect of KCN: Complete inhibition of the peroxidase occurred at 2×10^{-4} M KCN and 50 per cent inhibition at 5×10^{-6} M, which is in agreement with the results of Dixon and Elliott (1929), those of Hand (1933) with horseradish peroxidase, and those of Hussein and Cruess (1940) for grape peroxidase.

Effect of Sodium Azide: This was the strongest cauliflower peroxidase inhibitor tested by us, as it completely inhibited the enzyme activity at 10^{-5} M. This observation agrees with that of Keilin (1936), who found

that NaN_3 inhibited not only peroxidase but also phenolase and catalase.

Effect of Salts of Iron and Copper: Ferric chloride acted as an inhibitor at all concentrations tested, namely, 10^{-6} to 10^{-4} M; inhibition being 23 per cent at 10^{-4} M. Above 10^{-4} M the color of the $FeCl_3$ interfered with colorimeter readings.

At 7×10^{-4} M $CuCl_2$ caused a noticeable (about seven per cent) increase in activity and a slight inhibition (about five per cent) at 10^{-2} M, perhaps due to the chloride ion. The enhancing of peroxidase activity by very small concentrations of cupric ions is well known.

Effects of Other Salts: We found the chlorides of Ca and Mg inhibited peroxidase activity in similar degree at all concentrations, ranging from 10^{-7} to 1 M. There was no catalytic effect at high concentrations, as occurred with NaCl and NaBr. Smirnov (1925) had reported that the chlorides of the alkaline earths favor activity of peroxidase. In our tests evidently the inhibiting action of the chloride ion exceeded any enhancing effect of the Ca and Mg ions.

Sodium sulfide proved to be a very powerful inhibitor, closely paralleling KCN in that respect. $HgCl_2$ even at the very low concentration of 10^{-7} M, caused noticeable inhibition and completely inactivated the enzyme at 5×10^{-2} M.

Lithium chloride was either without effect on, or slightly enhanced, the enzyme's activity (at 8.2×10^{-3} M) in the range tested, namely, 10^{-6} to 1 M—differing markedly from NaCl and NaBr in this respect. Evidently the catalytic or enhancing effect of the Li ions counteracted the inhibiting effect of the Cl ions.

Regeneration of Cauliflower Peroxidase in Freezing Storage: Since peroxidase is very generally used in the frozen foods industry as an indicator of adequacy of blanching (scalding), its usefulness for that purpose would be greatly impaired if the enzyme regenerates itself during storage. For these reasons experiments were conducted to clarify this point.

The first recorded observation of the regeneration of peroxidase, in so far as we can ascertain, was made by Woods (1902) who observed that tobacco peroxidase reappeared some time after inactivation by heating. Others who made similar observations on peroxidase from other plants were Kulpsohn (1908), Biederman and Jernakoff (1924), Gallaghar (1924), Bach and Oparin (1925), Kern (1928), Pronin (1931), and Balls (1943). Bach and Oparin found that regeneration took place in the presence of molecular oxygen and an iron compound, and that a slightly alkaline pH was favorable. Kulikov and Babkon (1928) suggested that regeneration is due to elution of an adsorbed enzyme from colloids. Pronin observed that regeneration of heat-inactivated peroxidase is almost complete at pH seven.

Schwimmer (1944) stated that regeneration of heat-inactivated cabbage and turnip peroxidases is largely influenced by heating rate, has a positive temperature coefficient, and is a function of time. His data indicated the presence of several peroxidases in these vegetables. He suggested that regeneration involves redissolving of a heat-coagulated protein component and recombination with a soluble non-specific, prosthetic group.

Fischer (1943) and Herrlinger and Kiermeier (1944) have supported the adsorption and release theory.

In one series of our experiments five-c.c. portions of the purified enzyme extract at pH five were heated in thin-walled test tubes at 80°C. (176°F.) for 15, 30, 60, and 90 minutes, and cooled in an ice bath. An unheated control was also prepared. The tubes were stored at 0°C. (32°F.) and at —18°C.(—0.4°F.). In a second series cauliflower heads were trimmed and cut in pieces similar to sizes usually frozen commercially. These were heated at 80°C. for three, 10, 20, and 60 minutes; then cooled in cold running water, and stored together with untreated checks at 0 and —18°C.

The enzyme solutions were removed at intervals during a 60-day storage period, thawed at room temperature, shaken to resuspend heat-coagulated material which might contain a part of the protein fraction of the enzyme, and tested for activity, as previously described. The pieces were removed at intervals; thawed; ground with cold acetone; filtered; the residue air dried; reground with acetate buffer of pH five; filtered; and activity of the filtrate measured in the usual manner.

Data from the purified enzyme preparation were consistent, permitted plotting, and are shown graphically (Fig. 4). Attention is called to the fact that samples heated 15 minutes were diluted 15 to 1; the others were read without diluting.

Activity of the enzyme extracts, except for that heated 90 minutes, increased rapidly during the first three days' storage both at 0 and —18°C. That heated 90 minutes showed a somewhat greater lag, probably because the prolonged heating had more nearly permanently inactivated it.

After the initial rise in regeneration of activity there was a decline in several cases. In general, the shorter the period of heating the greater was the regeneration, although 15 minutes' heating was a notable exception.

The reason for the high initial regeneration rate followed by the decreased rate is not known, but a possible explanation based on the theory of Fischer (1943) is as follows: Upon heating, the enzyme is partially denatured, the protein fraction being precipitated. This precipitated protein absorbs and protects from heat some of the remaining undenatured enzyme. During the first few days of storage, the undenatured enzyme is eluted by some means from the denatured protein and again becomes active in solution. Then the denatured protein, according to the postulation of Schwimmer (1944), regenerates at a slow rate to give the further increase in peroxidase activity. The longer the blanch the greater the denaturation and the less is the possible regeneration of undenatured enzyme during the first few days of storage; this agrees with the observed results.

Extent of regeneration can be seen from the graphs, although it may be mentioned that the sample heated 30 minutes at 80°C. regained 85 per cent of its original activity in one month at —18°C.

Some of the data are difficult to explain, as there is considerable lack of correlation between length of blanching and extent of regeneration. This irregularity may have been due to irregularity in re-solubility of the

heat-coagulated proteins with which the enzyme was associated. Regeneration, as would be expected, was more rapid at 0 than at —18°C.

However, our observations very definitely indicate marked regeneration at 0 and —18°C. of cauliflower peroxidase inactivated at 80°C. It may be mentioned here that 80°C. was used instead of 100°C. because inactivation was too rapid at 100°C. to permit of well-spaced time intervals.

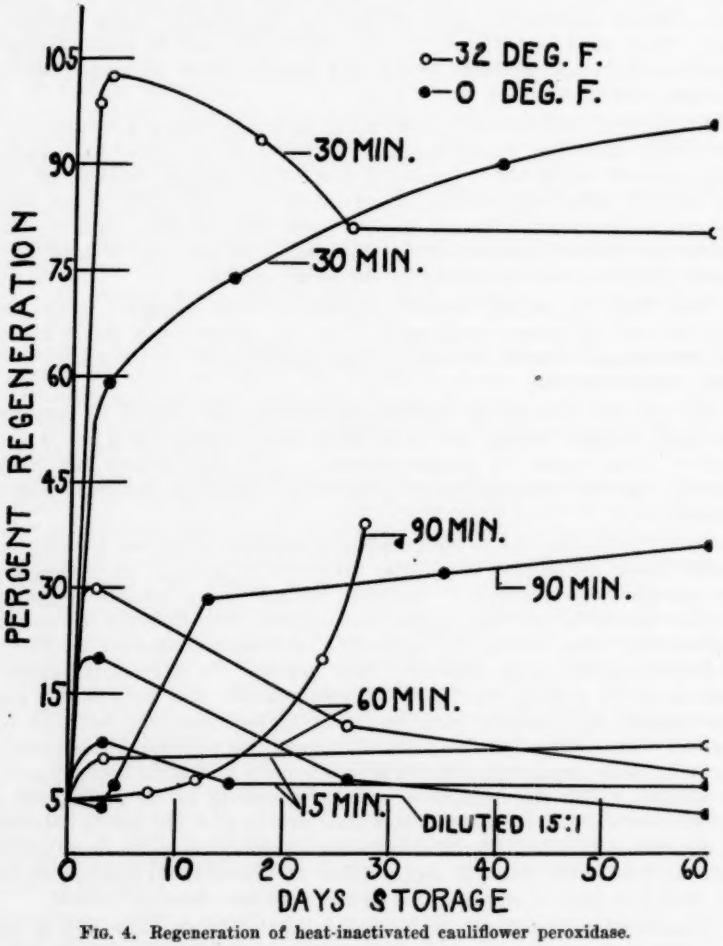

FIG. 4. Regeneration of heat-inactivated cauliflower peroxidase.

The amount of regeneration in the whole cauliflower was much less than in the enzyme extract. Three factors may account for this difference: First, there may be an enzyme inhibitor present in the cauliflower which is removed in the preparation of the extract. Second, the physi-

eal construction of the cauliflower may partially prevent the elution of adsorbed enzyme from precipitated protein or the recombination of denatured protein with its prosthetic group. Third, treatment of the ground solid tissues with acetone may have hindered redissolving of the heat-coagulated material.

Samples blanched three and 10 minutes showed significant regeneration at 0°C., and less at —18°C. For example, activity of the pieces heated three minutes at 80°C. was before storage 0.086, expressed as 2-log G. After 10 days at —18°C. it was 0.149, and at 109 days, 0.213. At 0°C. it was 0.213 at 10 days and 0.288 at 102 days. Results of observations on samples heated 10 minutes were less clear-cut, because of the high initial activity of the sample used to determine activity at zero days' storage. However, at 0°C. the activity increased from an initial of 0.312 to 0.498 in 102 days, and from 0.312 to 0.347 in 102 days at —18°C.

From our observations and those of other investigators we might wonder whether, if peroxidase in certain vegetables is only temporarily inactivated by blanching (scalding) before freezing storage, other enzymes may not exhibit a similar phenomenon. Such regeneration may account for development of off-odors and flavors by such enzymes in frozen vegetables blanched only sufficiently to barely inactivate peroxidase and other enzymes. Balls (1943), for example, states that uricase activity was regenerated on standing at room temperature after inactivation by heat.

Also, as peroxidase, in cauliflower at least, is regenerated in freezing storage, a positive test for peroxidase after such storage may not be a reliable indicator of blanching before storage.

Oxidation of Ascorbic Acid by the Ascorbic Acid Oxidase and the Peroxidase of Cauliflower: Ascorbic acid is universally distributed in plant tissues, and in the tissues of most plants is rapidly oxidized when they are damaged severely. Such oxidation may be due to: (1) The phenolase system, Johnson and Zilva (1937), which catalyzes the oxidation of certain ortho-hydroxy phenols by molecular oxygen to quinones, which in turn oxidize ascorbic acid. (2) The cytochrome system, Keilin and Hartree (1938). (3) Peroxidase, in a manner similar to that by phenolase except that H_2O_2 is required, Tauber (1936) and Huszak (1937). (4) Ascorbic acid oxidase, Kertesz *et al.* (1936) and Tauber *et al.* (1935), by direct oxidation of ascorbic acid using molecular oxidation. Non-enzymatically, inorganic copper will readily catalyze the oxidation of ascorbic acid, Ettori and Grangaud (1937).

Phenolase is not present in cauliflower and the cytochrome system was not included in our studies.

Ascorbic acid has been used in oxidase measurement by Neuweiler (1936), Ezell and Gerhardt (1940), Sreerangachar (1943), and more recently by Jimenez (1947), and by Ponting and Joslyn (1948).

In our experiments we made observations on the ability of our purified enzyme extract and of freshly expressed cauliflower juice to catalyze the oxidation of ascorbic acid by molecular oxygen (ascorbic acid oxidase) and by H_2O_2 (peroxidase).

We used for the measurement of ascorbic acid oxidase essentially the method described by Ponting and Joslyn (1948), which is based on determining by use of 2,6-dichlorophenolindophenol and the Evelyn colorimeter the ascorbic acid present in a buffered solution containing a known amount of ascorbic acid, and that remaining in the solution after a definite period of contact with a measured amount of the enzyme preparation or the natural juice under controlled conditions.

The oxidation of ascorbic acid by H_2O_2 catalyzed by peroxidase was measured colorimetrically as for ascorbic acid oxidase, except H_2O_2 and guaiacol were added approximately as outlined by Ponting and Joslyn (1948).

In order to fall within the range of the colorimeter, solutions must be quite dilute.

In our experiments a pH value of six was maintained for ascorbic acid oxidase measurements by means of phosphate-oxalate buffer and 5.38 for the peroxidase measurements, since it is nearer the optimum for peroxidase and there is less interference by ascorbic acid oxidase.

TABLE 2

Oxidation of Ascorbic Acid Catalyzed by Cauliflower Ascorbic Acid Oxidase and Peroxidase

Enzyme	Ascorbic acid oxidized mg./min. by one c.c. enzyme solution or juice
1. Ascorbic acid oxidase	
a. Enzyme extract	0.017
b. Fresh juice	1.000
2. Peroxidase	
a. Enzyme extract	0.350
b. Fresh juice	3.720
3. Totals	
a. Enzyme extract	0.367
b. Fresh juice	4.720

In a typical series of observations the results shown (Table 2) were obtained. We have estimated that one ml. of the natural juice used in our experiments oxidized ascorbic acid at the rate of 283 mg. per hour.

In our experiments the enzyme preparation and the fresh juice were allowed to act on the ascorbic acid for two minutes before the reaction was arrested by addition of oxalic acid. As mentioned previously, our technique for measurement of ascorbase oxidation of ascorbic acid is essentially that described by Ponting and Joslyn (1948), which is based on similar measurements by others; and similarly for measurement of peroxidase oxidation of ascorbic acid. In order to conserve space we have not presented details of these methods [the essentials are to be found in the reference mentioned above (1948), but we shall be glad to give details on request.]

Observations on the Natural Substrate of Cauliflower: Cauliflower frequently turns gray on the surface in freezing storage. It was thought that this discoloration might be caused by oxidation of a tannin or other

similar substrate by the oxidase of the cauliflower. Accordingly, leaves and cauliflower heads were disintegrated in acetone in a Waring Blendor and allowed to steep overnight; filtered; the filtrate evaporated under vacuum to remove acetone; and the residue then taken up in H_2O and tested qualitatively with the usual reagents, Allen (1923), for tannin. Tests were negative for the extract from the cauliflower heads and slightly positive for catechol tannin, for the extract from the leaves.

The extracts were further purified by fractional precipitation with neutral lead acetate; filtration; washing; suspension in water; and treatment with H_2S to precipitate lead. Filtrates from this mixture were concentrated to a syrupy consistency *in vacuo*.

This extract was diluted in H_2O and to it was added enzyme extract; also enzyme extract plus H_2O_2. After several days' storage in the cold room at 0°C. no darkening or graying had occurred. These experiments would indicate that enzymic oxidation of naturally occurring tannins is not responsible for the surface darkening of cauliflower, nor is the substrate of the leaves involved in this graying.

SUMMARY

Peroxidase activity was greatest in the vascular and epidermal layers of cauliflower stems. It was equally distributed throughout the parenchyma, but of much lower intensity.

A clear, very active enzyme solution was prepared by precipitation of the enzymes with acetone and re-solution in acetate buffer.

Rate of color development by the enzyme preparation mixed with dilute H_2O_2 and dilute guaiacol was used to measure peroxidase activity. Reasons are given for allowing the reaction to proceed in order to get the rate, rather than stop the reaction after a given period. Activity is expressed as 2-log G.

Phenolase was found absent from cauliflower tissue. Catalase was present in the raw cauliflower, but not in the purified enzyme extract. Ascorbic acid oxidase was present in both raw cauliflower and enzyme extract.

Up to a dilution of 15 to one the peroxidase activity of the enzyme extract was proportional to the concentration; at greater dilutions activity decreased at a greater rate than the dilution increased, possibly because of the increasing ratio of H_2O_2 to enzyme.

Optimum temperature range for the peroxidase was 30 to 34°C. and the thermal inactivation temperature about 90°C. for a five-minute holding period.

Optimum H_2O_2 concentration was found to be about 2.5×10^{-3} M.

Optimum pH range was found to be about 5.20 to 5.60, with a probable optimum at about 5.30.

Cuprie chloride and lithium chloride slightly increased the peroxidase activity; other chlorides decreased it. Fluoride was found very toxic and completely inhibited the enzyme at 3.5×10^{-2} M.

Peroxidase heat inactivated at 80°C. reappeared during subsequent storage of the extract and the cauliflower pieces at 0 and —18°C., the amount of regeneration being influenced by severity of the previous

heating. In some cases 30 to 100 per cent regeneration occurred. This observation is of industrial as well as theoretical importance, because the presence or absence of peroxidase in frozen vegetables is used as a criterion of adequacy of blanching.

An active ascorbic acid oxidase (ascorbase) was found present in the raw cauliflower juice and the purified extract. It was determined that one ml. of fresh cauliflower juice oxidized 283.2 mg. of ascorbic acid in one hour.

Both ascorbase and peroxidase were measured colorimetrically, using 2,6-dichlorophenolindophenol and an Evelyn colorimeter.

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ASCORBIC ACID CONTENT OF FROZEN AND CANNED FRUITS BEFORE AND AFTER PREPARATION FOR QUANTITY SERVING

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Factors which affect the ascorbic acid content of fresh fruits may also account for the variations in ascorbic acid content of the canned and the frozen fruits. According to several investigators, Hamner, Bernstein, and Maynard (1945); Todhunter (1938); Newman and Fellers (1940); Nagel and Harris (1943); Schroder, Satterfield, and Holmes (1943); and Kaski, Webster, and Kirch (1944), these factors are variety, size, degree of ripening, duration and temperature of storage, light intensity, day length, temperature and fertilizer, picking, processing, extraction, freezing, and glass and tin containers.

The comparative cost of serving frozen, fresh, and canned fruits as salads and desserts in a residence hall cafeteria was studied by Welch and Boney (1948). While fresh fruits in season were found to be lower in cost, canned fruits were higher than the frozen, with the exception of cherries and pineapple. These results suggested that determination of the ascorbic acid content of both frozen and canned fruits would be of value in considering an economical source of ascorbic acid for institutional use.

EXPERIMENTAL PROCEDURE

In the present study seven fruits—apples, apricots, blackberries, cherries, peaches, pineapple, and strawberries—were analyzed for total and reduced ascorbic acid content before and after preparation for quantity serving. The method of Roe and Oesterling (1944) was followed for the determination of total ascorbic acid with recoveries ranging from 96 to 106 per cent, while Loeffler and Ponting's (1942) method was used in determining the reduced ascorbic acid, with recoveries from 95 to 105 per cent.

Three different lots of each fruit, both canned and frozen, were prepared and served in Bruce Hall Cafeteria, North Texas State College, during March and April, 1948. Since canned strawberries were not available in institutional quantity, only frozen strawberries were used. All the fruits were obtained from local wholesale distributors. The canned fruits were obtained in No. 10 cans, while the frozen fruits were in five-, 10-, and 30-pound packages. The same size container of each fruit was used throughout the study.

An average size serving was considered to be a No. 16 dipper of apples, blackberries, cherries, crushed pineapple, and strawberries, or three slices

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of peaches, or three apricot halves. An average size serving was used as a sample, and three samples of each lot of canned and frozen fruits were taken for analysis. The frozen fruits were thawed according to directions on the package, and several packages mixed before taking samples. Three cans of a lot were opened, and contents mixed before samples were taken. Each sample was collected in a one-half-pint jar containing 50 ml. of one per cent metaphosphoric acid. The samples were mixed individually in the Waring Blendor, returned to the collection jars, and refrigerated until determinations for total and reduced ascorbic acid could be made.

Three samples, consisting of one serving each, of each lot of fruit after preparation for serving, were also taken, preserved, and analyzed in the same manner.

Two aliquots of each sample were analyzed as soon as possible after collection. If the two aliquots failed to check, three were analyzed and the average used for calculating the ascorbic acid content of the fruit.

DISCUSSION OF RESULTS

The standard-size portion described under Experimental Procedure was used in calculating the ascorbic acid value before the fruit was prepared for serving. When the fruits were used in pies and cobblers, the weight of the pastry was deducted from the total weight of the sample, so that only the weight of the fruit was used as the basis for comparing the ascorbic acid content in a serving. Therefore, each of the values reported represents the amount of ascorbic acid in a standard-size serving.

Reduced ascorbic acid was determined in order to compare the results with values reported by others. Reduced ascorbic acid was more difficult to determine in pigmented fruits since some of the color persisted even after the solution had been filtered with norit, thereby interfering somewhat with the color obtained when the dye was added to the solution. In spite of this interference, Kirk and Tressler (1941) found that an electrometric titrimeter method was satisfactory in analyzing pigmented fruits, vegetables, and their juices for ascorbic acid. They reported values of 0.40 to 1.04 mg./kg. for strawberries, 0.07 to 0.13 mg./kg. for peaches, and "very little" for blackberries and cherries. The amount of reduced ascorbic acid obtained on analyzing these fruits in the present study gave similar results.

In the cooking process some of the reduced ascorbic acid is changed to dehydroascorbic acid. Consequently, it was deemed advisable to determine the total ascorbic acid in these fruits since in this method all ascorbic acid present in the sample is converted to dehydroascorbic acid. The analyses (Table 1) show that the frozen fruits contained more total ascorbic acid before preparation for serving than did the canned fruits. Of the frozen fruits, apricots gave the highest average total ascorbic acid value (18.0), and apples the lowest (4.2), for an average serving. Of the canned fruits, the highest total ascorbic acid was found in pineapple (5.6); the lowest, in peaches (1.2). Some of the lots of frozen fruits showed a wide variation in their average total ascorbic acid content, namely, 7.4 to 33.5 mg. for an average serving of blackberries and 9.4 to 14.4 mg. for peaches. Others,

TABLE 1

Reduced and Total Ascorbic Acid Values Per Serving of Frozen and Canned Fruits Before and After Preparation

Fruit		Total ascorbic acid						Reduced ascorbic acid					
		Frozen			Canned			Frozen			Canned		
Kind	Lot	Before	After	Difference	Before	After	Difference	Before	After	Difference	Before	After	Difference
		mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Apples (cobbler)	I	3.9	5.0	+1.1	1.4	2.3	+0.9	0.5	0.0	−0.5	0.5	0.0	−0.5
	II	4.4	4.5	+0.1	2.5	1.9	−0.6	0.2	0.0	−0.2	0.5	0.5	0.0
	III	4.4	5.5	+1.1	1.2	1.2	0.0	0.0	0.0	0.0	0.5	0.5	0.0
	Av.	4.2	5.0	+0.8	1.7	1.8	+0.1	0.2	0.0	−0.2	0.5	0.3	−0.2
Blackberries (cobbler)	I	8.1	2.6	−5.5	1.8	1.2	−0.6	0.0	0.0	0.0	2.0	0.0	−2.0
	II	7.4	1.6	−5.8	1.1	1.6	−0.5	0.0	0.0	0.0	1.4	0.5	−0.9
	III	33.5	3.7	−29.8	2.0	2.0	0.0	0.0	0.0	0.0	1.3	0.5	−0.8
	Av.	16.3	2.6	−13.7	1.6	1.6	−0.4	0.0	0.0	0.0	1.5	0.3	−1.2
Apricots (salad)	I	19.7	17.6	−2.1	1.6	1.4	−0.2	0.0	0.0	0.0	0.0	0.0	0.0
	II	17.8	15.2	−2.6	2.5	1.5	−1.0	0.0	0.0	0.0	0.0	0.0	0.0
	III	16.3	17.9	+1.6	1.4	1.4	0.0	0.0	0.0	0.0	0.0	0.6	+0.6
	Av.	18.0	16.9	−1.1	1.8	1.4	−0.4	0.0	0.0	0.0	0.0	0.2	+0.2
Peaches (salad)	I	14.4	11.3	−3.1	1.3	1.1	−0.2	4.0	4.1	+0.1	0.4	0.4	0.0
	II	9.4	7.5	−1.9	1.1	1.1	0.0	0.0	0.0	0.0	1.2	0.0	−1.2
	III	9.6	5.0	−4.6	1.2	1.2	0.0	0.0	0.0	0.0	0.5	0.5	0.0
	Av.	11.1	7.9	−3.2	1.2	1.1	0.0	1.3	1.4	0.0	0.7	0.3	−0.4
Cherries (pies)	I	13.2	4.5	−8.7	0.9	0.7	−0.2	0.6	0.6	0.0	0.3	0.4	+0.1
	II	10.0	5.6	−4.4	1.1	0.9	−0.2	0.7	0.8	+0.1	0.3	0.4	+0.1
	III	11.6	4.1	−7.5	2.1	0.9	−1.2	0.7	0.7	0.0	0.0	0.3	+0.3
	Av.	11.6	4.8	−6.9	1.4	0.8	−0.6	0.6	0.7	0.0	0.2	0.4	+0.2
Pineapple (pies)	I	7.1	5.7	−1.4	5.3	8.9	+3.6	0.5	0.6	+0.1	0.8	0.5	−0.3
	II	9.2	4.9	−4.3	5.3	6.6	+1.3	0.0	0.6	+0.6	0.5	0.5	0.0
	III	8.0	5.0	−3.0	6.2	6.9	+0.7	0.0	0.5	+0.5	0.6	0.6	0.0
	Av.	8.1	5.2	−2.9	5.6	8.1	+1.5	0.2	0.6	+0.4	0.6	0.5	−0.1
Strawberries (fruit cup)	I	14.9	11.1	−3.8	.....	.....	.....	1.3	0.6	−0.7	.....	.....	.....
	II	10.0	6.1	−3.9	.....	.....	.....	1.0	0.6	−0.4	.....	.....	.....
	III	12.5	8.9	−3.6	.....	.....	.....	1.1	0.6	−0.5	.....	.....	.....
	Av.	12.5	8.7	−3.8	.....	.....	.....	1.1	0.6	−0.5	.....	.....	.....

however, showed little variation. In all canned fruits ranges for total ascorbic acid were much less than in the frozen.

Values for reduced ascorbic acid were low for both the frozen and the canned. Frozen blackberries and apricots and the canned apricots had none. Only frozen strawberries and canned blackberries gave slightly better than one mg./serving. One lot of the frozen peaches and one of the frozen pineapple contained reduced ascorbic acid; whereas, all lots of the canned fruit, with the exception of one of cherries, gave a value for reduced ascorbic acid. On the basis of the fruits analyzed in this study, all were poor sources of reduced ascorbic acid.

All frozen fruits except apples gave lower values of total ascorbic acid after preparation for serving. No explanation for this is known since all the blackberries served as cobbler showed a marked decrease in total ascorbic acid. All frozen fruits were better sources of ascorbic acid after preparation than any of the canned, with the exception of the canned pineapple, which showed an increased ascorbic acid value after preparation as pies; whereas, only one of the lots of apples gave the increase for cobbler. Both frozen and canned pineapple gave similar values after preparation, 5.2 and 8.1, respectively. In all other instances frozen fruits were superior in total ascorbic acid to the canned, prior to and following preparation for serving.

TABLE 2

Percentage[1] of Total and Reduced Ascorbic Acid From Frozen Fruits as Compared With Canned Fruits, With Frozen Fruit Used as Control

Fruit	Total		Reduced	
	Before	After	Before	After
	pct.	*pct.*	*pct.*	*pct.*
Apples	60	64	150[2]	30[2]
Apricots	89	97	.....	.....
Blackberries	90	38	.....	.....
Cherries	88	83	67	43
Peaches	89	86	46	80
Pineapple	31	58[2]	200[2]	17

[1] To obtain this percentage the average amount of ascorbic acid in the canned fruit was subtracted from the average ascorbic acid value of the frozen fruit. The difference was divided by the average ascorbic acid of the frozen fruit and multiplied by 100. [2] Percentage grater for canned fruit.

Method of preparing the fruit for serving had little to do with change in ascorbic acid value, although the fruit prepared as salads showed less change than for the other methods, even fruit cup. While the frozen fruits lost more ascorbic acid than the canned during preparation, the ascorbic acid value was always higher for the frozen fruits.

The comparative ascorbic acid values, total and reduced, are given (Table 2). When the ascorbic acid of the frozen fruit was used as the control, it was evident that total ascorbic acid values for all frozen fruits were higher than for the canned 31 to 90 per cent before preparation. After preparation, all frozen fruits, with the exception of pineapple, were higher 38 to 97 per cent. In the case of pineapple, the greater amount is probably due to its higher initial reduced ascorbic value (200), the reduced having been converted to dehydroascorbic during preparation.

It is to be noted that the canned apples had more reduced ascorbic acid than the frozen both before and after preparation. However, when these percentages are compared with the average values (Table 1), it is evident these per cents are not significant, as the actual milligram values are negligible.

These data support the evidence that ascorbic acid values of frozen-pack fruits are superior to the canned. From this, together with the results of Welch and Boney's study (1948), it may be concluded that frozen fruit may be advantageously used for institutional service.

SUMMARY

Total and reduced ascorbic acid contents were determined on seven fruits, both frozen and canned apples, apricots, blackberries, cherries, peaches, pineapple, and frozen strawberries.

Frozen and canned fruits were compared for total and reduced ascorbic acid values before and after preparation. In all cases, with exception of pineapple, the frozen fruit was higher than the canned in total ascorbic acid content after preparation.

Average retentions of total and reduced ascorbic acid after preparation for quantity serving were higher for canned fruits, but the initial content was lower, so that the frozen fruit actually was superior to the canned.

No relationship was found between method of preparation and retention of total and reduced ascorbic acid. The widest range of variation was in fruits prepared in cobblers; the least variation, in those prepared as salad.

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CHARACTERISTICS OF COUNTRY-STYLE HAMS AS RELATED TO SUGAR CONTENT OF CURING MIXTURE [1]

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Salt is an effective agent in dehydrating meat and thus, in time, causes the meat to become dry and hard. Sugar and molasses are considered useful in counteracting this hardening effect and at the same time imparting a more desirable flavor to the cured meat, Helser (1929), Warner (1938), Tomhave (1925), Ziegler and Miller (1938), and Moulton (1929).

Lewis (1937) stated that while sugar may soften the brashness of the salt, it does not impart a sweet flavor to cured meats except in the case of bacon. In the cooking of bacon sugar concentration is markedly increased due to rendering of the fat. Commercially cured hams analyzed by Lewis contained, on an average, less than one-half of one per cent of sucrose. Lewis also reported that in blind taste tests of corn sugar, second sugars, honey, molasses, maple-flavored cane sugar, and no-sugar cures the sugar used or the absence of it produced no apparent flavor differences.

Urbain (1944) pointed out that ordinarily cane or beet sugar is used for meat curing. Use of corn sugar is limited because it tends to darken the meat in cooking and because its use in large quantities promotes excessive bacterial action and consequent color destruction.

Haldane (1901), Jensen (1935), Lewis (1936), Brooks (1937), and Greenwood, Lewis, Urbain, and Jensen (1940) have emphasized the importance of sugar, particularly reducing sugar, in the development of the desired color in cured meats. Moulton (1935) stated it is desirable to have reducing sugars present to provide an environment suitable for acid fermentations and to prevent putrefactive fermentation.

Greenwood *et al.* (1940) and Urbain and Jensen (1940) have stressed the importance of avoiding excessive amounts of reducing sugars during the curing process. This condition lowers the pH to the extent that oxidation of the nitric oxide hemoglobin is favored, with resulting color destruction.

Considerable difference of opinion exists among authorities as to the advantages of adding sugar to a curing mix for the production of southern, country-style hams. While generally recommended curing formulas contain from 10 to 30 per cent sugar, nevertheless the finest Virginia- and Tennessee-type hams are cured without sugar. For this reason, and for the reason that previously reported work indicates there is considerable question as to the necessity of using sugar as an ingredient in the curing

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mixture, it was deemed advisable to secure information on the characteristics of hams cured with varying proportions of sugar.

METHODS

Twenty hogs of uniform size, and having similar feeding and breeding histories, were slaughtered for this experiment. The average hog carcass weight was 151 pounds, with a hot dressed yield of 78 per cent (shipper style). The basic curing formula consisted of eight pounds of salt and three ounces of saltpeter per 100 pounds of meat. A total of eight cures was prepared consisting of zero, six, 11, 20, 27, 33, 38, and 43 per cent sucrose. The hams were divided into eight lots of five each. The green hams weighed an average of 13.66 pounds. To eight pounds of salt and three ounces of saltpeter sufficient sugar was added to give the percentage of sugar previously listed for each of the curing mixtures. The hams were cured two and one-half days per pound and overhauled on the fourth and 11th days. Following completion of the curing process they were soaked three hours in tap water 13°C.(55°F.) and scrubbed in hot water 49°C. (120°F.). After washing they were dried two hours at 54°C.(130°F.), then smoked for 22 hours at 54°C. Following smoking, the hams were held in controlled temperature storage. The average mean temperature was 20°C.(68°F.), with temperatures fluctuating between extremes of 17 and 22°C.(63 and 72°F.). The ordinary minimum and maximum temperatures were 19 and 21°C.(67 and 69°F.), respectively. During the first two weeks of storage the relative humidity was not controlled, and varied between 85 and 90 per cent. Because heavy mold growth developed on the surfaces of the hams it was necessary to lower the humidity. This was accomplished by using a reheat coil in connection with the evaporative cooler. With this method of control the usual relative humidity range was between 50 and 55 per cent, with a maximum range of 15 per cent. At mid-term of the storage period the relative humidity was again allowed to reach its own level, which approximated 75 and 80 per cent. This again resulted in heavy mold growth and the humidity was returned to the controlled level.

Moisture, fat, salt, and "glucose" determinations were made at three different periods, namely, 24 hours after the completion of smoking, after 26 days' storage, and after 125 days' storage. Cores three-fourths of an inch in diameter were taken through the thick muscles of the ham, approximately one inch from the posterior end of the *ischium* (aitch bone). After removal of the cores the fat from the skin side was trimmed and the lean portion remaining divided into three equal-size pieces. These three sections were termed, in order from the aitch-bone side to the skin side, as the inside, middle, and outside. On the initial sampling, one ham from each lot was cored and analyzed, while at the end of the 26- and 125-day storage periods every ham was cored and analyzed. All core openings were filled with a low-melting-point, plasticized wax to protect the exposed surfaces.

Moisture determinations were made by drying the samples 16 to 20 hours at 70°C.(158°F.), followed by five hours' drying in a vacuum oven (28 inches) at the same temperature. Fat determinations were made

according to the official A.O.A.C. (1945) method, except each sample was ground in a mortar with Hyflo Super-Cel, prior to extraction with ether. Salt content was determined by disintegrating the fat-free sample in a Waring Blendor with 100 ml. of water and titrating potentiometrically with silver nitrate an aliquot acidified with nitric acid. "Glucose" was determined on the dried, fat-free samples by the Micro Method for Reducing Sugar, A.O.A.C. (1945), except the inversion process was carried out at room temperature according to the official A.O.A.C. (1945) method. At the completion of the aging period (125 days), center slices were broiled according to the standard method listed in *Meat and Meat Cookery* (1942) and taste-tested by a Palatability Committee.

RESULTS

Average per cent weight losses during curing and subsequent aging are shown (Table 1). These figures are approximate indices of the moisture losses, which are seen to be marked over the whole period of study. Initially, weight losses at the 38 and 43 per cent sugar levels appear to have been somewhat higher than for the lower sugar levels, but this difference was not maintained throughout the storage period.

TABLE 1

Average Weight Losses in Hams During Curing and Storage

Treatment	Sugar level—Per cent of curing mixture							
	0	6	11	20	27	33	38	43
	pct.	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*
Loss in weight during curing	6.5	7.9	7.7	7.2	6.1	7.1	9.0	8.5
Total loss in weight through smoking	9.8	10.7	9.7	10.1	8.9	9.7	12.3	11.1
Total loss in weight after 26 days' storage	14.6	15.5	15.1	14.6	13.2	14.4	16.2	15.0
Total loss in weight after 125 days' storage	24.2	25.1	24.1	24.2	23.0	25.5	24.7	24.3

Moisture, fat, salt, "glucose," and pH determinations after completion of the 26- and 125-day aging periods were summarized (Table 2). Initial values for one ham from each treatment, on all characteristics except for the pH determinations, are also listed. It will be noted there was a marked decrease in moisture content as storage was extended. This decrease resulted in an increase in salt concentration. There was some tendency for "glucose" to decrease with time at the higher sugar levels. However, all "glucose" values listed, with one exception, are less than one-half of one per cent. The pH values listed (Table 2) were consistently higher at 125 days than at 26 days. This shows that an appreciable decrease in acidity occurred between the 26th and 125th day of storage.

Regression equations relating the several characteristics under study to the sugar level of the curing mix are presented (Table 3). These are for the whole core. It will be noted (see Tables 2 and 3) that increasing the sugar concentration, while initially having no significant[4] effect on

[4] The term "significant" is used in the sense of being statistically significant at the five per cent level and highly significant at the one per cent level.

moisture content, was significantly effective in reducing salt content and increasing "glucose" content. After 26 days' storage, the increased sugar level, in addition to significantly reducing salt content and increasing "glucose" content, had a highly significant effect in reducing moisture content. At the end of 125 days' storage the sugar level still had a significant effect on decreasing salt content, but not on the moisture and other characteristics studied.

TABLE 2

Average Chemical Analysis of Hams for Each Sugar Level

Length of storage	Sugar level—Per cent of curing mixture								Five per cent L.S.D.[1]
	0	6	11	20	27	33	38	43	
	Per cent moisture								
Initial	61.37	65.34	58.98	54.77	62.18	62.22	55.98	62.08	12.96
26 days	59.57	57.11	56.77	54.94	56.73	57.00	53.50	53.20	3.32
125 days	49.99	48.35	46.92	44.99	49.47	47.39	44.19	47.12	4.44
	Per cent fat								
Initial	7.89	5.22	10.31	16.64	9.47	8.60	12.40	7.83	12.62
26 days	8.35	8.76	10.11	9.31	10.10	8.88	10.38	11.54	3.53
125 days	9.60	11.07	13.16	13.63	11.07	10.61	12.92	12.41	4.11
	Per cent salt								
Initial	4.97	3.08	3.90	3.44	3.14	2.26	3.20	2.36	2.03
26 days	4.07	4.02	4.73	4.67	3.31	3.86	3.81	3.22	1.12
125 days	5.58	5.48	6.03	5.76	4.50	4.41	4.90	4.01	1.28
	Per cent "glucose"								
Initial	0.17	0.33	0.21	0.35	0.47	0.34	0.48	0.35	0.29
26 days	0.21	0.37	0.28	0.46	0.42	0.35	0.60	0.46	0.14
125 days	0.24	0.32	0.25	0.33	0.27	0.24	0.46	0.28	0.12
	pH values								
26 days	5.77	5.78	5.66	5.66	5.72	5.75	5.70	5.64	0.15
125 days	6.05	5.94	5.78	5.79	5.87	6.08	5.89	5.90	0.23

[1] Least significant difference for sugar at the five per cent level.

Regression equations (shown Table 3) were also computed for inside, middle, and outside separately. From these equations it was possible to predict values for the various periods at specific sugar levels. The predicted values for moisture, fat, salt, and "glucose," as related to position in the ham, storage period, and for the zero and 43 per cent levels are listed (Table 4). Values for the intermediate levels can be secured by linear interpolation. In considering the zero sugar level, the inside or exposed surface of the ham consistently had a lower moisture content than the middle and outside sections. There was a marked decrease in moisture content of all three sections as storage progressed. As each section of the ham lost moisture, the relative proportion of fat at each location increased accordingly, but the inside section always had the lowest fat content. Lack of uniform salt distribution in the hams at the onset of the storage period was very pronounced. As the storage period progressed, however, there was a marked movement of salt to the lesser exposed sections. This movement continued until, after 125 days, the inner section had the lowest salt concentration. That the low salt

concentration of the inside section after 125 days' storage is closely associated with the reduced moisture content can be ascertained by the brine ratio (per cent salt/per cent moisture). The brine ratios on this basis for the inside, middle, and outside were, respectively, for zero per cent sugar: 0.127, 0.129, 0.111; and for 43 per cent sugar: 0.098, 0.097, and 0.087. In the main, "glucose" distribution was similar to salt distribution for the different sections at the various periods.

TABLE 3

Regression of Moisture, Fat, Salt, "Glucose," and pH Values of Whole Hams on Per Cent Sugar in Curing Mixture

Characteristic	Initial values	26-day storage	125-day storage
Moisture[1]	61.41 −.047[2]	58.53−.109[2,4]	48.71−.064[2]
Fat[1]	8.73 +.048[2]	8.59+.049[2]	11.09+.032[2]
Salt[1]	3.39 −.043[2,3]	4.42−.021[2,3]	5.92−.039[2,4]
"Glucose"[1]	.226+.005[2,3]	.27+.006[2,4]	.26+.002[2]
pH[1]	.....	5.75−.002[2]	5.92−.004[2]

[1] Characteristic, i.e. moisture, fat, etc. [2] Level of sugar in curing mixture. [3] Regression coefficient significantly different from zero. [4] Regression coefficient highly significantly different from zero.

Changes in moisture, fat, and salt content for the three sections at the 43 per cent sugar level are highly similar to those described for the zero per cent sugar level. The principal difference between the zero and 43 per cent sugar levels was that moisture values were significantly lower with the higher sugar level after 26 days' storage. On the average, this relationship held, also, for the other sugar levels. One interesting observation in the case of the 43 per cent sugar level was that the relatively high "glucose" content of the inside section continued to be maintained even after 125 days' storage.

A study of the palatability scores indicates the Testing Committee was unable, on the average, to note differences in the hams that could be ascribed to the various sugar levels. It was the opinion of the Committee that while a few of the very high sugar level hams had a tendency to be a little "off" in flavor, nevertheless, on the average they were all very acceptable. The Committee also noted the hams were extremely uniform in appearance, aroma, firmness, texture, saltiness, tenderness, juiciness, and flavor. While color of the lean was not rated by the Committee, it was the authors' opinion that all the hams had a color which was desirable, as well as characteristic, for this type of cure.

DISCUSSION AND CONCLUSIONS

In a discussion of the use of sugar in meat curing, it is necessary to differentiate between two principal types of cures. First, there is the short commercial cure, which is mild-flavored, moist, and perishable, and must be stored under refrigeration. Second, there is the country-style cure, which is saltier, drier, and must be stored or aged for several months at high temperatures to complete the curing process and to develop the desired flavor characteristics.

Investigations reported in the literature indicate sugar may have an important function in curing meat, not only as a preservative but also

TABLE 4

Predicted Average Values Showing Relationship of Moisture, Fat, Salt, and "Glucose" Content to Position in Ham at the 0 and 43 Per Cent Sugar Levels and After Storage

Characteristic	Position	Initial		26 days		125 days	
		0 pct. sugar	43 pct. sugar	0 pct. sugar	43 pct. sugar	0 pct. sugar	43 pct. sugar
Per cent moisture	Inside	58.0	53.7	53.0	45.9	38.7	37.6
	Middle	61.8	64.9	60.3	57.2	52.6	46.5
	Outside	64.4	59.5	62.4	58.4	54.9	53.9
	Overall	61.4	59.4	58.5	53.8	48.7	46.0
	L.S.D. 5 pct.[1]	4.2	4.1	3.2	3.0	4.2	4.0
Per cent fat	Inside	4.5	7.7	6.5	7.6	8.1	8.1
	Middle	10.2	8.9	9.6	11.6	13.0	15.5
	Outside	11.5	15.9	9.7	13.0	12.1	13.9
	Overall	8.7	10.8	8.6	10.7	11.1	12.5
	L.S.D. 5 pct.[1]	6.2	6.0	3.5	3.2	4.5	4.3
Per cent salt	Inside	3.5	4.9	5.2	4.0	4.9	3.7
	Middle	3.2	1.5	4.5	3.7	6.8	4.5
	Outside	1.2	.8	3.5	3.0	6.1	4.7
	Overall	3.4	2.4	4.4	3.5	5.9	4.3
	L.S.D. 5 pct.[1]	1.1	1.0	.7	.7	1.1	1.0
Per cent "glucose"	Inside	.34	.86	.32	.75	.24	.41
	Middle	.19	.23	.28	.49	.28	.28
	Outside	.15	.23	.21	.29	.26	.30
	Overall	.23	.44	.27	.51	.26	.33
	L.S.D. 5 pct.[1]	.14	.13	.10	.10	.09	.09

[1] Least significant difference at the five per cent level.

in color fixation. It cannot be presumed, however, that color fixation will not proceed satisfactorily without the addition of sugar to the cure. Greenwood *et al.* (1940) have pointed out it is desirable to have reducing sugars available early in the curing period with all cures, and especially with short cures. The point emphasized was that reducing sugars insure the presence of reduced hemoglobin to react with the nitrite and, therefore, give an additional assurance of proper color fixation. Whether the inversion of sucrose takes place at a rate sufficiently rapid to insure the presence of adequate reducing sugar early in the curing process is somewhat outside the scope of this paper. The question is whether or not, with the long cure, there is sufficient justification for adding this rather expensive ingredient to the curing mixture. Commercially, and on the farm, millions of pounds of hams and shoulders are being cured each year without sugar, in preference to the sugar cure.

While sugar may play an important role in the curing process, nevertheless the results of this experiment support the view that it is not an essential ingredient in the production of high quality, aged, country-style-cured hams. From a practical operations viewpoint, there are also fewer problems with microbial growth in curing rooms when the content of sugar is minimized. It should be pointed out, however, that the data provide

no evidence that reasonable amounts of sugar are in any way injurious to the quality of cured meat. It was apparently not possible to differentiate the sugar-cured from the non-sugar-cured hams by palatability tests.

Other experimental results that are of especial interest are concerned with the distribution of salt, "glucose," and moisture in the three sections, as related to length of storage period. Salt equalization results are of the same general order as reported by Ziegler and Miller (1938). The low "glucose" content of the cured meat is in agreement with the work reported by Lewis (1937).

In conclusion, it may be stated that the proportion of sucrose in the cure has no over-all marked effect on the aged, long-cured, country-style hams, from either a chemical or a taste standpoint. Effects of sugar were most pronounced at the onset of the storage period and became increasingly less important as storage progressed. Initially, the increased levels of sugar were effective in increasing the "glucose" content, and reducing the salt and moisture contents. At the end of 125 days, however, the sole effect of the sugar was limited to the salt content. Associated with the prolonged aging was a marked reduction in the hydrogen ion concentration, but sugar level had no effect on this characteristic. As storage time progressed the distribution of salt became more nearly equalized. The close relationship of salt content to moisture content was marked in the three sections of the ham.

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EVALUATION OF FUTURE PERISHABILITY OF INTACT FRESH EGGS BY RADIO-FREQUENCY CONDUCTIVITY

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In a previous communication, Romanoff and Hall (1944), it was shown that high-frequency conductivity of the intact egg increases with age of the egg and provides an index of the progressive deterioration that time produces in the egg's contents.

Since it is well known that strictly fresh eggs are not of uniform quality, as assessed by candling and various organoleptic and laboratory procedures, the present investigation was undertaken to ascertain the possible value of radio-frequency conductivity readings for detecting internal differences in new-laid eggs.

These differences in quality are correlated with the relative perishability of eggs in storage. It was therefore considered worthwhile to include in this study further inquiry into the changes in the electrical conductivity of held eggs, with the objective of determining what relationship, if any, exists between initial and final conductivity.

MATERIALS AND METHODS

Eggs used were laid by a flock of White Leghorn hens one to two years old. Conductivity readings (in arbitrary units) were made with an apparatus operating on the same principle, Romanoff (1944), as one previously described, Romanoff and Cottrell (1939) and Romanoff and Frank (1941). It was equipped, however, with a quickly read device to compensate for the weight of the egg, and was designed as a small portable model for field use.

RESULTS

It was observed that eggs one day old differ greatly in electrical conductivity. Average readings for 12 or more eggs from each of 137 hens varied over a wide range.

Table 1 shows the frequency distribution of these average readings, a total of 2,033 eggs having been used in this trial.

It also became clear that the eggs of each hen were of fairly uniform conductivity. Some hens consistently produced "good" eggs—that is, eggs of low conductivity — whereas other hens laid "poor" eggs with equal regularity.

Ninety-five eggs laid on a single day were used in the storage experiment. The initial conductivity of each egg was determined within 24 hours after the time of laying, and the entire lot of eggs was then held at 21°C. (70°F.). Readings were taken at intervals of one week for 12 weeks. Conductivity of all eggs increased during this period, at a rate directly proportional to the original conductivity. The conductivity of the best

and the poorest single egg is shown graphically (Fig. 1), as well as the curves of the averaged readings for the 10 best eggs, for the 10 poorest eggs, and for the entire lot.

TABLE 1

Frequency Distribution of Intact Fresh Eggs, and of Hens Laying These Eggs, According to Radio-Frequency Conductivity

Conductivity readings	Frequency distribution		
	Eggs examined	Hens laying these eggs	
groups	*No.*	*No.*	*pct. of flock*
0.36-0.40	53	5	3.6
0.41-0.45	186	17	12.4
0.46-0.50	372	33	24.0
0.51-0.55	665	64	46.7
0.56-0.60	167	16	11.9
0.61-0.65	20	2	1.4
Totals	2,033	137	100.0

Due to the differing rates at which conductivity increased, readings at the end of the 12-week holding period varied over an even wider range than at the beginning, Romanoff and Romanoff (1949). In addition, the lowest readings obtained at the end of the experiment were the same as the highest readings recorded when the eggs were new-laid. Conductivity data for the fresh and the held eggs are given (Table 2).

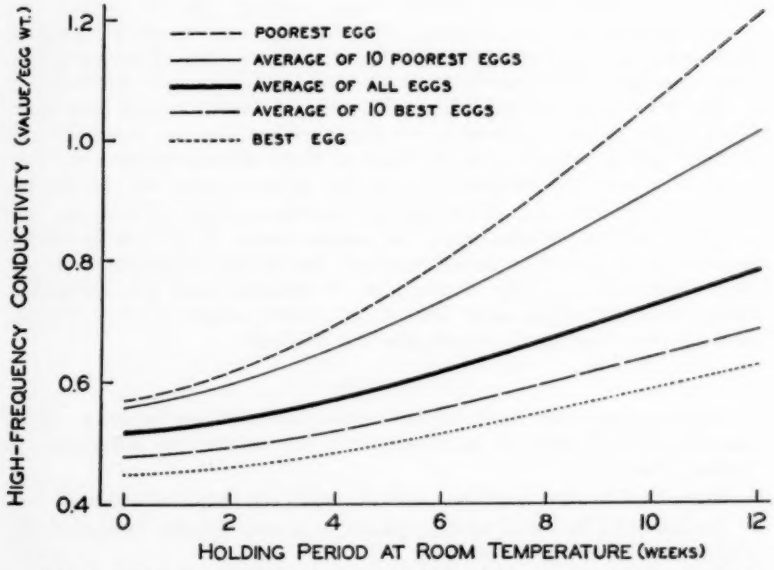

FIG. 1. Changes in radio-frequency conductivity of intact eggs during storage at 21°C.(70°F.).

TABLE 2

Frequency Distribution of Intact Eggs According to Radio-Frequency Conductivity When Fresh and After Being Held 12 Weeks at 21°C.(70°F.)

Conductivity readings	Frequency distribution	
	Fresh eggs	Eggs held 12 weeks
groups	*pct.*	*pct.*
0.31-0.40	1.1	.....
0.41-0.50	38.9	.....
0.51-0.60	57.9	.....
0.61-0.70	2.1	29.5
0.71-0.80	.....	38.9
0.81-0.90	.....	18.9
0.91-1.00	.....	4.2
1.01-1.10	.....	5.3
1.11-1.20	.....	2.1
1.21-1.30	.....	1.1

DISCUSSION

The above results appear to indicate that internal differences, of an undetermined nature, exist in new-laid eggs and are readily revealed by variations in radio-frequency conductivity. In view of the relationship already noted, Romanoff and Hall (1944), between the conductivity and the condition of held eggs, it seems fair to infer that the quality of fresh eggs is equally well correlated with their conductivity. Reasons for these correlations are not yet clear.

The observation that individual hens produce eggs of consistently similar conductivity is of interest. This finding, also, tends to denote a relationship between conductivity and quality, since eggs of the same hen closely resemble each other in their internal condition when new-laid.

The more rapid increase in conductivity of the held eggs that gave the initially higher readings opens for consideration the possibility of selecting eggs for storage on the basis of their original relative conductivity (if a practical electronic device for doing so could be perfected).

No attempt was made to explore the possible correlations between conductivity and various commonly used measurements of the quality of the opened egg. It should be stated, however, that it was impossible to detect blood spots. Although the conductivity of chicken blood is considerably higher than that of albumen, and almost twice as high as that of yolk, blood spots are too small to influence the readings.

CONCLUSIONS

New-laid eggs differ greatly in radio-frequency conductivity. It is suggested that differences in conductivity are related to differences in internal quality.

Eggs laid by the same hen are of fairly similar conductivity.

Conductivity increases as time passes, at a rate directly related to the original conductivity of the egg.

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PALATABILITY OF KALE IN RELATION TO COOKING PROCEDURE AND VARIETY

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Kale is a green leafy vegetable [belonging to the cabbage family *(Brassica)*] which deserves wider recognition. It can be grown easily and is resistant to frost, so it need not be harvested until late fall or early winter, even in colder sections of the country.

Kale is also high in nutritive value. In common with other green, leafy vegetables, it contributes large amounts of vitamin A (as carotene), riboflavin, and iron, an appreciable amount of thiamine, and, in addition, considerable amounts of ascorbic acid and calcium. However, it is difficult to increase the consumption of a foodstuff on the basis of nutritive value alone. If the product is not appetizing after cooking, it will not be eaten. Hence, a knowledge of method of cooking is as important as a knowledge of nutritive value.

The primary objective of the investigation reported here was to determine the relative palatability of four varieties of kale, when cooked in covered and uncovered pans, in varying amounts of water, for various lengths of time, when cooked in a pressure saucepan, and when steamed.

EXPERIMENTAL PROCEDURE

Palatability tests were conducted in the spring season of 1944 and the fall seasons of 1944 to 1947, inclusive.

Except for the first season, the kale was grown by the Horticulture Department at the University of Connecticut in plots arranged in randomized blocks. It included four varieties: Dwarf Blue Scotch Curled, Dwarf Green Scotch Curled, Tall Green Scotch Curled, and Siberian. Dwarf Blue is the variety most commonly found in the markets. The kale was picked early in the morning; leaves which were not fully mature were selected. In preparation for cooking, the leaves were stripped from the midrib and broken into approximately one-inch pieces. They were weighed into 125-gm. portions, an amount which ordinarily would constitute about four servings. They were then washed and dried between towels at room temperature.

In general, the laboratory procedures followed those recommended by the Committee to Standardize Methods of Cookery for the National Cooperative Project on the Conservation of the Nutritive Values of Foods (1942). The kale was cooked by boiling, pressure-cooking, and steaming. In earlier tests it was boiled for three different periods in three amounts of cooking water, in both covered and uncovered pans. The kale was turned at the middle of the cooking period. Preliminary tests were conducted to determine the time for pressure-cooking and steaming.

The following cooking times were used in most tests:

Method	Description	Minutes
Boiling, covered	Short	4
	Medium	6
	Long	9
Boiling, uncovered	Short	6
	Medium	9
	Long	13.5
Pressure-Cooking (15 lb.)	Short	0 or ¼
	Medium	½
Steaming	Short	8
	Medium	12

In boiling, the amount of water was adjusted so that after cooking there remained 25, 312, and 600 grams for the small, medium, and large amounts, respectively.

Relative palatability was determined by tasting panels, numbering from four to 30 for any single test. The panels comprised students and staff in Home Economics and students in Horticulture at the University of Connecticut.

The treatments were arranged in factorial designs with six to nine samples in any one test. Each taster ranked the samples in order of preference, ties not being allowed. Nine samples seemed to tax the capacity of the tasters, but fewer than six would have reduced the efficiency of the experiment. In each test samples were presented in a random order, but could be retasted at will. To determine the stability of the judgments, many tests were repeated, either without change or in different combinations. As well-marked preferences were established, or differences shown to be entirely unimportant, the later experiments could be simplified. Hence, over the four years covered by the experiment attention was centered progressively on preferred varieties and methods of cooking.

METHOD OF ANALYSIS

Before analyzing results statistically, the ranks were transformed to the scores given by Fisher and Yates (1948) for normalizing ordinal data. This facilitated the estimation of relative palatability in complex experiments, Bliss, Anderson, and Marland (1943).

The method of analysis will be illustrated with results for one season (1946). Variables in the five tests involved were two cooking times and three varieties. The long cooking time had rated low in earlier tests, so that only the short and medium cooking times were used in this season, with uncovered pans. Since the Tall Green variety had not been liked, it was omitted from the plantings. The three remaining varieties, each cooked for two different periods, required only six samples, which comprised a complete test. An early snow in the fall of 1946 prevented more than five tests. Sixteen of the tasters were present throughout and the analyses are restricted to their ratings.

Total transformed scores for each day's test are shown (Table 1). The

error term in each row is a composite of the various interactions with taster (i.e., taster x time, taster x variety, and taster x time x variety). Scores for the individual tasters for each of the five dates were totalled and are shown (Table 2). Here the interaction of sample with date provided a separate error term for judging the significance of differences in the total scores for each taster.

The combined analysis is shown (Table 3), in which the direct effects from differences in the total scores and their first- and second-order interactions are isolated. The analysis shows there was no distinct preference

TABLE 1

Total Scores of Palatability Tests on Three Varieties of Kale Cooked by Boiling in Medium Amount of Water, Two Lengths of Time, in Uncovered Pans

[Arranged by Dates (Fall, 1946)]

Date	No. of tasters	Cooking time			Variety			
		Short (6 min.)	Medium (9 min.)	Significance[1] of difference	Dwarf Blue	Dwarf Green	Siberian	Significance[1] of difference
1946								
Nov. 1	16	—5.44	5.44	20 pct.	5.71	—19.92	14.21	**
15	16	6.09	—6.09	20 pct.	—3.99	—8.29	12.28	*
22	16	—.63	.63	.....	7.64	—14.40	6.76	**
Dec. 6	16	5.37	—5.37	.....	1.51	3.78	—5.29	.....
13	16	1.26	—1.26	.....	6.47	—3.78	—2.69	.....
Grand total.....		6.65	—6.65	.....	17.34	—42.61	25.27	**

[1]As assessed by analysis of variance.

TABLE 2

Total Scores of Palatability Tests on Three Varieties of Kale Cooked by Boiling in Medium Amount of Water, Two Lengths of Time, in Uncovered Pans

[Arranged by Tasters (Fall, 1946)]

Taster	No. of tests	Cooking time			Variety			
		Short (6 min.)	Medium (9 min.)	Significance[1] of difference	Dwarf Blue	Dwarf Green	Siberian	Significance[1] of difference
1	5	—1.04	1.04	.....	4.01	—4.45	.44	20 pct.
2	5	—2.74	2.74	.....	—2.10	—4.05	6.15	*
3	5	3.66	—3.66	20 pct.	—1.28	1.47	—.19	.....
4	5	—1.27	1.27	.....	1.47	.44	—1.91	.....
5	5	—3.37	3.37	20 pct.	—1.07	—6.57	7.64	**
6	5	1.50	—1.50	.....	1.70	—4.01	2.31	20 pct.
7	5	.05	—.05	.....	—.67	—7.60	8.27	**
8	5	—4.65	4.65	**	4.05	—8.71	4.66	**
9	5	6.96	—6.96	**	3.82	—1.66	—2.16	20 pct.
10	5	2.53	—2.53	.....	5.73	1.91	—7.64	**
11	5	—1.46	1.46	.....	1.87	—.59	—1.28	.....
12	5	1.65	—1.65	.....	1.07	—.67	—.40	.....
13	5	6.52	—6.52	**	.63	—4.89	4.26	*
14	5	1.48	—1.48	.....	—5.92	.59	5.33	*
15	5	—2.74	2.74	.....	4.66	—3.82	—.84	20 pct.
16	5	—.43	.43	.....	—.63	0.00	.63	.....
Grand total.....		6.65	—6.65	.....	17.34	—42.61	25.27	**

[1]As assessed by analysis of variance.

TABLE 3

Analysis of Variance of Total Scores (16 Tasters) of Palatability Tests on Three Varieties of Kale Cooked by Boiling in Medium Amount of Water, Six and Nine Minutes, in Uncovered Pans on Five Dates (Fall, 1946)

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Total	400	330.0000	.....	.....
Cooking time	1	0.3685	0.3685	0.61
Variety of kale	2	17.2179	8.6090	14.34**
Time x variety	2	0.2088	0.1044	0.17
Time x tasters	15	22.4208	1.4947	2.49**
Variety x tasters	30	56.8109	1.8937	3.15**
Time x variety x tasters	30	20.6080	0.6869	1.14
Time x date	4	3.6941	0.9235	1.54
Variety x date	8	22.9741	2.8718	4.78**
Time x variety x date	8	5.5664	0.6958	1.16
Remainder (error)	300	180.1305	0.6004	1.00

for either six or nine minutes of cooking time, although the significant interaction of time by tasters testified to some lack of agreement with respect to cooking time. Choice of cooking time was not influenced either by variety of kale or date of experiment, since none of the interactions of time by variety, time by date, time by variety by taster, and time by variety by date was significant. Differences in variety, on the other hand, had a marked effect upon relative palatability, as shown by the significance of the direct effect. This also varied with the taster (variety x taster) and with date of test (variety x date), although not with length of cooking time, as has been noted.

Although variations in form and extent of the data modified the statistical analysis in the several seasons, the same general plan was followed throughout.

RESULTS AND DISCUSSION

Methods of Cooking

Boiling: Early tests indicated clearly that kale cooked in the smallest amount of water was not liked by the tasters; whereas, there was no preference between the two larger amounts of water. Since considerably more ascorbic acid and thiamine were lost to the cooking water (data from this laboratory) when the large amount was used, the medium amount was chosen for all later tests.

Relatively long cooking was not liked as well as cooking for either of the shorter periods. However, preferences were not so marked as with the amount of cooking water and were subject to greater variation both from taster to taster and from season to season. Some of the tasters preferred the kale cooked a short time, others a medium time, and still others a long time. Obviously, results cannot be interpreted wholly in terms of the totals, but must take account of individual preferences. There was some indication of a seasonal trend in the preferred cooking time. Longer times were preferred earlier in the season and the shorter times later. Freezing which the kale underwent in the field apparently tended to make the leaves more tender, but this was not evident in the short season of 1946.

With a limited number (six) of comparisons of kale cooked in the covered and uncovered pans, no significant differences were found in scores.

Pressure-Cooking: Preliminary tests indicated that pressure-cooking up to 15 lb. and for 30 seconds at 15 lb. was comparable to boiling for six and nine minutes. Judging from the total score for 23 tasters, boiling was preferred to pressure-cooking, but all tasters did not agree with one another, some preferring the pressure-cooked kale.

Steaming: Steaming for eight and 12 minutes was found to be comparable to boiling for six and nine minutes. The tasters showed such a definite dislike for steamed kale that it was included in only a few tests.

Varieties

By the middle of the third season it was evident the tasters did not like Tall Green Scotch Curled kale, so it was not tested further. Of the remaining three varieties, Dwarf Blue Scotch Curled and Siberian were preferred to Dwarf Green Scotch Curled. In the 1945 and 1946 seasons Siberian was rated higher than Dwarf Blue, but in the 1947 season this preference was reversed. As noted before, the tasters differed from one another in their likes and dislikes, not only in the 1946 season (shown Tables 2 and 3) but in the other seasons as well. Some preferred Dwarf Blue, some Siberian, and other tasters liked the two varieties about equally well. None, however, showed a significant preference for Dwarf Green. On a single day there was more unanimity of opinion in relation to variety than either to length or method of cooking.

In 1946 there was evidence of a seasonal fluctuation in varietal preference, the preference for Siberian diminishing through the season. This may have been due in part to the fact that the Siberian kale was infected with powdery mildew, even though no damaged leaves were picked and the kale was thoroughly washed.

So far as performance in the field was concerned, Dwarf Blue was superior to Siberian. It gave much higher yields and seemed to withstand cold weather better. In these respects Dwarf Green was similar to Dwarf Blue. Tall Green stands high, as the name implies, and seemed to be more affected by weather than the other varieties. An early, heavy frost sometimes turned its leaves yellow. Leaves of the Dwarf Blue, Dwarf Green, and Tall Green are quite similar. They are all very curly, differing primarily in shade of green. Leaves of Siberian differ in shape and are often veined in purple or red, a characteristic which becomes more noticeable as the season advances.

Comments

This study indicates that for greatest acceptability, kale should be boiled in an uncovered pan, in a medium amount of water, for the relatively short time of six to nine minutes. This amount of water is enough to immerse the kale to about one-half its depth, and the kale must be turned when it is half-cooked. However, preferences differ and method of cooking may be adjusted to suit the individual. Both pressure-cooked and steamed kale retained considerably more (in some cases all) of their vitamin C and thiamine values. If the sulfur compounds which are reputedly formed in the covered pans are not objectionable in flavor, or do not cause digestive

upsets, pressure-cooking might be used. Steamed kale was disliked so heartily it can scarcely be recommended. Nearly all ascorbic acid and thiamine lost from the boiled kale was recovered in the cooking water. If the cooking water were to be used in soups, gravies, etc., or drunk as a beverage, these losses would be minimized.

It was evident from inspection of the data and from recorded comments that the tasters were not influenced equally by the various characteristics of the kale. Some gave more attention to its texture, others to flavor. To some, time of cooking was important; to some, variety; to some, cooking method; and to some, all factors were important.

Kale can be picked through a relatively long season in late fall and early winter, until covered by snow. As the season advances and the kale freezes it seems to become sweeter in flavor and, as has already been noted, more tender. Then, within the space of a week, it may develop a distinct hay-like off-flavor. During the season of 1944-45, for example, a sample of kale picked January 4 was unusually sweet and good-flavored; a sample picked January 11 was distinctly hay-like in flavor, and inedible. Usually it could not be picked after the middle of December because of the heavy snow cover. Kale will often winter-over and make a light growth in the spring which can be used for early greens.

SUMMARY AND CONCLUSIONS

The relative palatability of four varieties of kale cooked by boiling, pressure-cooking, and steaming was determined by approximately 20 tasters, over a period of five seasons.

Boiling in an uncovered pan for six to nine minutes, when covered with water to about half its depth, was generally preferred, but individual tastes varied. Some liked longer cooking; some liked kale cooked in the pressure saucepan. Steamed kale was generally disliked.

Dwarf Blue Scotch Curled and Siberian were preferred to Dwarf Green Scotch Curled or Tall Green Scotch Curled.

There was some indication of seasonal trends both for cooking times and for varieties. Longer cooking times were sometimes preferred early in the season before the kale had been subjected to much freezing weather. In one year the rating of Siberian kale diminished during the season.

Kale might well be used through late fall and early winter in colder sections of the country as a dietary source of vitamin C and other nutrients.

ACKNOWLEDGMENT

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USE OF MILK FAT FRACTIONS IN BAKED PRODUCTS[1]

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In the course of experimentation to determine the physical and chemical properties of glyceride fractions separated from milk fat, Henderson and Jack (1944), certain differences were observed that made it desirable to determine the practicability of using some of these fractions as shortening agents in certain food products. Consequently, the experiments to be reported here were undertaken.

Although butter is frequently used in the preparation of cakes, its use in pastry has been limited by the fact that butter, as such, has a relatively poor shortening power in pastry, in spite of its high percentage of unsaturated glycerides, Lowe (1944). In this respect, it should be noted that Denton and Lowe (1944) observed that moisture-free butter, drained free of curd, produced a very tender pastry. The use of milk fat fractions, free of protein, would be of particular value in the diet of individuals who are allergic to milk and its products.

EXPERIMENTAL PROCEDURE

Milk Fat Fractions: The fractions were prepared by precipitation from solvent (Skelly Solve A) at —20 and —53°C. The following table shows the chemical characteristics of each fraction:

TABLE 1

Chemical Characteristics of Milk Fat Fractions

	Saponification No.	Iodine No.
—20° ppt.	236	13.4
—53° ppt.	240	32.8

Thus, it can be seen that the —20° precipitate was considerably firmer at room temperatures than the —53° precipitate.

Cakes: Two methods were used to prepare the experimental cakes. The first was the conventional method, in which fat and sugar are creamed together first and the dry ingredients then added alternately with the liquid. The second method was the quick method, in which the fat and two-thirds of the liquid are added to the dry ingredients and beaten for two minutes. The egg and remaining liquid are added and the mixture beaten for two more minutes. When the fat was in a liquid state, it was added 45 seconds after the first addition of milk. Typical recipes for these

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two methods were chosen and proportions used are indicated in the following table:

TABLE 2

Ingredients for Quick and Conventional Method Cakes

	Fat	Eggs	Sugar	Cake flour	Milk	Baking powder	Salt
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Conventional	30	48	100	96	80	4	3
Quick	30	33	120	79	80	4	2

A simple scoring system was devised to compare the experimental cakes with the standard cakes on the basis of appearance, flavor, texture, volume, tenderness, and moistness. Two standard cakes were used, one prepared with butter and the other with a commercial shortening agent (Spry). These two products were of comparable quality under the conditions of our experiment.

It was found that the milk fat fractions required considerable manipulation. The —20°ppt. was a hard, brittle solid at room temperature, whereas, the —53°ppt. was a liquid at room temperature and had to be refrigerated before it could be creamed with sugar.

Since these two milk fat fractions did not yield satisfactory cakes as compared to the standard, it was decided to try to improve their performance by the addition of an emulsifier (Tween 61). An attempt was made to determine the optimum amount of emulsifier.

In the experimental procedure, an effort was made to approximate home baking conditions. Ingredients were weighed on a trip balance and were used at room temperature, except where the difficulty of manipulation of the fat required it be refrigerated or heated. Batters were mixed in a Mix Master electric mixer at low speed. The mixing technique was standardized as much as possible. The cakes were baked in seven-inch round pans in a gas oven at 177°C.(350°F.). After they were baked, they were allowed to stand for 15 minutes in the pan before removal.

Pastry: The pastries were studied with two methods, the conventional (ice water) method, and the hot water method. Thirty grams of fat were cut into 56 grams of all-purpose flour with a pastry blender. Twenty grams of ice water, about 4°C.(39°F.) were then added, the dough rolled into a ball and refrigerated 15 minutes. It was then rolled out on a pastry cloth and baked 14 minutes at 475°F. in an electric oven.

Since the —20°ppt. was not plastic at room temperature, it was necessary to use a hot water method. Boiling water was poured over the fat and this mixture added to the flour. After refrigeration for 15 minutes, it was rolled and baked as described above.

Standard pie crusts were prepared with a commercial shortening (Snowdrift) and with lard, since these are preparations commonly used for pastry. Some pie crusts were made with butter, for purposes of comparison. The emulsifier was used in a few instances with the milk fat fractions. A simple score sheet was used to judge results.

Cookies: A few brief experiments were carried out on cookies, also. Four different types of cookies were selected for experimental purposes. These included rolled, drop, pan, and refrigerator cookies.

In all cases, sugar and fat were creamed together, beaten eggs added, and the dry ingredients and liquid, if any, added at the last. Drop cookies were dropped from a teaspoon onto an ungreased baking sheet and baked in an electric oven at 350°F. 14 minutes.

Rolled cookies were rolled out on a pastry cloth to one-quarter-inch thickness. They were cut and baked on an ungreased cooky sheet at 375°F. 11 minutes. The refrigerator cooky dough was shaped into a long roll, wrapped in waxed paper, and refrigerated 24 hours at 18°C. (64°F.). The cookies were then cut, and baked 12 minutes at 375°F. Pan cookies were spread about one-quarter-inch thick in a greased, square pan and baked at 350°F. 30 minutes.

Recipes for these products are as follows:

TABLE 3

Recipes for Cookies

	Fat	Sugar	Eggs	Flour	Baking powder	Soda	Salt	Liquid
	gm.	*cup*	*gm.*	*cup*	*tsp.*	*tsp.*	*tsp.*	
Refrigerator	50	¼	12	½	None	⅛	1/16	None
Pan	33	⅓	32	½	⅓	None	⅛	None
Rolled	50	½	48	1¼	1	None	¼	1½ tsp. milk
Drop	33	¼	24	½	None	1/16	⅛	½ tsp. water

Standard cookies were prepared with butter as the shortening agent.

RESULTS

Conventional Method Cakes: After some experimentation with the —20°ppt., it was found best results were obtained if the fat was melted, then allowed to resolidify until slightly above (29°C.) room temperature before creamed with sugar. However, when compared with standard cakes made with butter or Spry, the cake made with —20°ppt. was definitely of inferior quality. For this reason, it was decided to try the effect of an emulsifier (Tween 61).

The fat was melted with the emulsifier, cooled to about 29°C., then creamed with the sugar, as before. The resulting product was definitely superior to the product made with —20°ppt. alone. It was found that the volume of the cake varied directly with the amount of emulsifier used. The optimum amount of emulsifier was found to be 1.5 grams (five per cent in relation to fat used). In order to give a fineness of texture equivalent to the standard cake, it was necessary to use 30 grams (37.5 per cent) more milk when the emulsifier was used. This cake (—20°ppt., five per cent emulsifier, and 37.5 per cent more milk as variation in recipe) proved to be as acceptable as the standard cakes.

Cake made by the conventional method with —53°ppt. had to be made with the fat refrigerated to a solid state, since it is a liquid at room temperature. This cake was superior to cake made with —20°ppt. alone, but inferior to the standard cake. When cakes were made with —53°ppt., with 1.5 grams of emulsifier and 20 grams (15 per cent) of extra milk (optimum for this fat fraction), they were superior to those made with —53°ppt. alone, but did not compare well with cakes made with —20°ppt. and emulsifier.

Quick Method Cakes: If it was melted first, the —20°ppt. gave a better quick method cake than conventional method cake. However, it was again observed that a better product was made with —20°ppt., 1.5 grams of emulsifier, and a 37.5 per cent increase in milk in the recipe.

Unexpectedly, since it is a liquid at room temperature, the —53°ppt. did not give by the quick method a cake which was acceptable. The texture was coarse and the cake tough. Some improvement was noted if the fat was refrigerated until solid. The addition of 1.5 grams of emulsifier and 20 grams more milk improved these cakes considerably, but they still did not compare with the cakes made with —20°ppt. and emulsifier (quick method).

Pastry: In agreement with previous investigators, we found that lard makes the most desirable pie crust and that Snowdrift was also very acceptable. Pie crust made with butter had an excellent flavor, but was tough and less flaky.

Pie crust made with —20°ppt. by the conventional (ice water) method was completely unacceptable, since it was tough, not flaky, and very greasy. A reduction in amount of fat used (to 25 grams) did not influence results. Some improvement was noted if 20 grams of —20°ppt. was melted and allowed to resolidify to 29°C. to make it more plastic. However, this pie crust could not be considered acceptable. Therefore, it was decided to try a hot water method.

Boiling water proved to be more satisfactory than hot water, 77°C. (171°F.), and for this method the optimum level of fat was found to be 25 grams. This pastry proved to be of very good quality and as acceptable as the standard. As might be expected, the addition of emulsifier had little effect on the outcome, since the use of fat in pastry does not depend on its emulsification.

The —53°ppt. made an acceptable pastry if it was refrigerated at 7.8°C.(46°F.) before used with the ice water method.

Cookies: The most successful cookies made with the milk fat fractions were the rolled and the drop cookies. Here again, the chief difficulty was in the manipulation of milk fat fractions. In all cases it was necessary to melt the fat and then resolidify it to 29°C. The —53°ppt. was refrigerated to 7.8°C., followed by quick manipulation to prevent it from melting while the dough was being mixed. The drop and rolled cookies were indistinguishable from those made with butter when the fats were handled in the manner described above. Pan cookies were acceptable, but somewhat drier and crisper than those made with butter. This could be adjusted by alterations in the amount of liquid in the recipe.

Difficulty of manipulation of the fat was most obvious in the preparation of refrigerator cookies. The —20°ppt. resulted in a dough too hard to slice after refrigeration. Those made with —53° ppt. were too soft to be handled easily. In no case were refrigerator cookies made with milk fat fractions as acceptable as those made with butter.

SUMMARY

The use of two milk fat fractions, precipitated from solvent at —20°C. and —53°C., as shortening agents in baked products has been studied.

It was found that the —20°C. fraction resulted in a satisfactory cake, whether prepared by conventional or quick method, if five per cent of an emulsifier (Tween 61) was added to the fat. It was also necessary to use 37.5 per cent more milk than in the standard recipe. The —53°C. fraction is less satisfactory as a shortening agent for cake, in spite of the addition of emulsifier to it.

The —20° precipitate cannot be used for pastry if the conventional ice water method is used. However, it does make an excellent hot water pastry. The —53° precipitate makes an acceptable pastry if chilled to 7.8°C., to make it plastic enough to use in the conventional method. The emulsifier was not found to have any effect on quality of pastry produced with these milk fat fractions.

In brief experiments with cookies, it was observed that both fractions, if carefully manipulated, result in acceptable products, with one exception: Refrigerator cookies are impractical with these fractions, because the physical state of the fats at the temperatures used does not permit adequate manipulation of the cookies.

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EFFECT OF SUGARS ON OXIDATION OF ASCORBIC ACID. I. KINETICS OF AUTO-OXIDATION OF ASCORBIC ACID[1]

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It has been established, during the past 10 years, that sugars exercise a protective effect on the non-enzymic and enzymic oxidation of ascorbic acid, Joslyn (1949), but the mechanism and extent of this effect are largely unknown. In the course of our investigation of this effect, it became necessary to establish the conditions of oxidation of ascorbic acid used as a base for the evaluation of the specific effects of sugars. Although the chemistry of ascorbic acid has been well established, Haworth and Hirst (1939), and the general course of its oxidation is known, none of the intermediates has been isolated in chemically pure form and there is still some confusion in the literature on the mechanism of the auto-oxidation. While most of the recent investigators agree that the auto-oxidation of ascorbic acid in acid solutions is catalyzed by Cu^{++}, there is still disagreement on the catalytic effect of Fe^{+++} and other metals, and there is no agreement on the kinetics or the rate-determining step in the reaction, Keilie and Zilva (1935), Barron, de Meio, and Klemperer (1936), Mack and Kertesz (1936), Dekker and Dickinson (1940), Sinohara (1940), Schümmer (1940), Hand and Greisen (1942), Steinman and Dawson (1942), Silverblatt, Robinson, and King (1943), Peterson and Walton (1943), and Weissberger, Lu Valle, and Thomas (1943, 1944). In these investigations, the rate of auto-oxidation usually was measured by rate of oxygen uptake in Warburg respirometers, and largely either in unbuffered solutions or in buffers of various types.

Barron *et al.* (1936), and Peterson and Walton (1943), gave their original data in the form of observed half-life of the reaction. Rate constants derived from observed half-lives are in general not as precise as rate constants derived from a set of measurements made during the course of the reaction. Sinohara (1940) studied the rate of oxidation in dilute ascorbic acid solution through which air was bubbled, but followed the course of oxidation by iodometric determination of hydrogen peroxide produced in the reaction. Schümmer (1940) used dilute ascorbic acid solutions, but depended on the oxygen dissolved in his solutions. Dekker and Dickinson (1940) studied the copper-catalyzed oxidation of ascorbic acid in perchloric acid solutions containing relatively small amounts of ascorbic acid, so that continual oxygenation was not necessary except in one case. Temperatures at which measurements were made also differed, 20 to 30°C.(68 to 86°F.), and intercomparison of results is difficult because of the high temperature coefficient of the reaction. Although it has

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been established that in the copper-catalyzed oxidation of ascorbic acid, approximately two atoms of oxygen are used per mol of ascorbic acid (in comparison with one atom of oxygen per mol of ascorbic acid in enzyme-catalyzed oxidation)—Hand and Greisen (1942)—direct comparisons between initial rates determined by titration of residual ascorbic acid—Mack and Kertesz (1936) and Dekker and Dickinson (1940)—with those in which oxygen uptake alone was measured are difficult to make. Since satisfactory methods of determining even the first product of oxidation—dehydroascorbic acid—are not yet available, it is not possible to study the course of the reaction by a combination of oxygen uptake, ascorbic acid loss, and oxidation-product formation.

The most detailed of the recent studies were those of Weissberger *et al.* (1943, 1944). They investigated the kinetics of the auto-oxidation of 0.008 molar ascorbic acid by measuring the rate of oxygen uptake under carefully controlled conditions at 20°C.(68°F.) Weissberger, Lu Valle, and Thomas (1943) investigated the auto-oxidation of l-ascorbic acid in the presence of sufficient cyanide and thiocyanate to suppress the catalytic action of metals at pH from 4.7 to 9.2 in phenolsulfonate (pH 7.8 to 9.2) and phosphate (pH 4.7 to 7.6) buffers. Under these conditions, they reported that both the monovalent and the divalent ion of ascorbic acid participate, the divalent ion reacting 10^5 faster than the monovalent ion with oxygen at atmospheric pressure. When the partial pressure of oxygen is reduced to one-fifth, reaction rate of the divalent ion is reduced in proportion to the oxygen concentration, but that of the monovalent ion is not. These authors suggested that monovalent ion undergoes a slow process before it reacts with oxygen. In the absence of cyanide and thiocyanate and in the presence of copper, they found that the reaction rate of the monovalent ion was proportional to the oxygen concentration. Weissberger and Lu Valle (1944) investigated the auto-oxidation of 0.008 molar ascorbic acid solution at pH 2.6 to 9.3 in the presence of copper nitrate, added in concentrations ranging from 4×10^{-6} to 200×10^{-6} mols per liter in 0.2 M potassium phosphate, potassium hydrophthalate, sodium acetate, and sodium phenolsulfonate buffers. At very low copper concentrations they found the rate to increase faster than the concentration of the metal, but at higher concentrations the rate increase was proportional to increase in copper concentration. Rate of the copper-catalyzed oxidation of ascorbic acid was found to vary in a complex manner with pH and type of buffer. This they interpreted to be due to complex formation of some of the buffers with the copper catalyst. They interpreted their results to indicate that neither the neutral l-ascorbic acid nor the divalent ion but only the monovalent ion is subject to copper catalysis. A concentration of 2×10^{-5} mols/liter of copper increased reaction rate of the monovalent ion by a factor of 10^4.

Experiments described in the present paper were carried out for the purpose of better establishing the order of the reaction, to compare the auto-oxidizability of the more common isomers, to obtain the temperature coefficient, and to study particularly the effect of pH and copper content in buffers known to form complex copper compounds to varying degrees.

MATERIALS

Ascorbic Acid: Ascorbic acid used was that of the Hoffmann-La Roche Company and was a racemic mixture of l-ascorbic acid. It was assumed to be 100-per cent pure.

Solutions: The buffered solutions were made from shelf reagents.

The acid component of the buffer was made up in 0.1 M concentration in the case of the orthophosphate, citric acid, and oxalic acid buffers. A plot of pH versus volume of 0.1 M NaOH was determined for each of the acids. The buffers were then made up from the acid and 0.1 M NaOH by preserving the ratio of volume of acid to volume of NaOH at the pH desired. The 0.1 M citric acid + 0.2 M sodium dihydrogen phosphate buffered solutions were made by following the instructions for McIlvaine's Standard Buffer Solutions, McIlvaine (1921). Total concentration of the acid constituents of these buffers thus varied with pH (shown Table 1). It was found subsequently that this affected the rate of oxidation, as will be discussed later.

Each stock solution was analyzed for copper and iron content. Copper was determined by the Marsh (1941) modification of the Coulson (1937)-

TABLE 1

Composition of Buffers Used

A. H_3PO_4—NaOH buffer on basis of 1,000 ml. completed solution

pH	ml. 0.1 M H_3PO_4	ml. 0.1 M NaOH
7.50	345	655
6.70	378	622
5.80	454	546
4.45	483	517
3.70	493	507
3.00	521	479

B. $H_2C_2O_4$—NaOH buffer on basis of 1,000 ml. completed solution

pH	ml. 0.1 M $H_2C_2O_4$	ml. 0.1 M NaOH
7.00	300	700
5.10	310	690
4.00	358	642
3.10	455	545

C. HPO_3—NaOH buffer on basis of 1,000 ml. completed solution

pH	ml. 0.2 M HPO_3	ml. 0.1 M NaOH
7.00	408	592
5.80	500	500
5.00	553	447
4.00	571	429
3.00	611	389

D. H Cit.—NaOH buffer on basis of 1,000 ml. completed solution

pH	cc. 0.1 M H Cit.	cc. 0.1 M NaOH
6.59	246	754
5.87	267	733
4.97	329	671
4.02	432	568
3.09	625	375

Drabkin (1939) procedure, which consisted of measuring the intensity of sodium diethyldithiocarbamate complex with copper in amyl acetate solution. Iron was determined by a photoelectric colorimetric modification of the Saywell and Cunningham (1937) procedure, cf. Fortune and Mellon (1938), which measured the color intensity of the ferrous orthophenanthroline complex. Stock solutions were prepared of the orthophosphate, citrate, and oxalate buffers. The metaphosphate buffers were prepared immediately before use.

Water: Distilled water prepared in a tin-lined still was used in all cases. The water was analyzed for copper and iron, as just described.

Copper and Iron Solutions: Copper solutions used for adjusting the copper content of the solutions were prepared by dissolving N.B.S. copper in a small portion of nitric acid and diluting with water to the calculated volume. The iron solutions were made by dissolving pure iron wire in 6 N nitric acid and diluting to volume.

Indophenol: The 2,6-dichloroindophenol used for determining amount of ascorbic acid in the solutions was from the LaMotte Company. It was prepared as a stock solution of 0.07 gram per 100 ml. water. This solution was diluted one to 10 when used for titration. The stock solution was stored at —0.56°C.(32°F.) and made up fresh every five days, or oftener.

EXPERIMENTAL PROCEDURE

In the oxidative procedure 50 mg. of ascorbic acid were weighed into 100-ml. volumetric flasks, the required amount of solution added, the mixture shaken for from 15 to 20 seconds, about 70 ml. poured into a straight-sided glass jar (11 cm. high and four cm. in diameter, and fitted with a sintered glass, six mm. i.d. tube with plug 2.5 cm. long and 1.2 cm. in diameter), and the whole placed in a bath maintained at —1.11°C.(30°F.). Oxygen from a cylinder was then passed through the bubbler continuously during the reaction. Oxygen flow was measured with a manometer type flow meter and the flow maintained at 100 ml. per minute. Timing of the reaction was begun when the solution was first shaken. The pH on the remainder of the solution was then taken within five minutes of the start of the run on a Beckmann industrial model pH meter.

Samples were removed from the reaction vessel at intervals and pipetted at once into a two-per cent solution of metaphosphoric acid. Titration for residual ascorbic acid was then performed by the method of Bessey and King (1933), which consisted of titration of the metaphosphoric acid solution with 2,6-dichloroindophenol. The indophenol was standardized daily by titration against a known amount of ascorbic acid in two-per cent metaphosphoric acid. The residual ascorbic acid was calculated as mg. per cent remaining in the solution.

RESULTS OF RATE MEASUREMENTS

Order of the Reaction: Data in the literature on the order of the reaction with respect to ascorbic acid concentration are conflicting. Weissberger *et al.* (1943, 1944) express their results as first-order constants. Peterson and Walton (1943) report the reaction to be first-order for 50 to 80 per cent of the reaction below pH seven and above pH 11. Silverblatt *et al.* (1943) state, "It is evident that the rate constants show a

strong dependence on initial concentrations of ascorbic acid," but they calculate their results as first-order constants. Dekker and Dickinson (1940) use first-order constants, but note a drift in the constant. Barron *et al.* (1939) described the reaction as zero order; Schümmer reported the reaction to be pseudo-unimolecular.

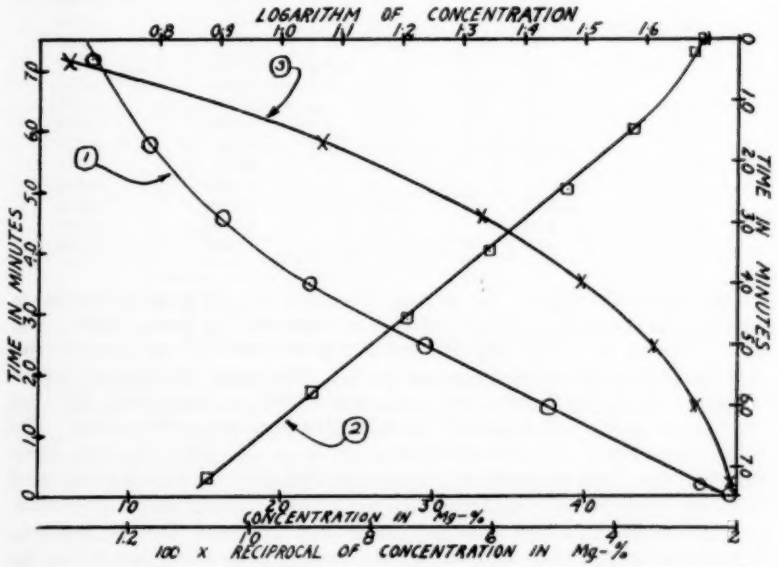

FIG. 1. Typical auto-oxidation of ascorbic acid (phosphate buffer, pH 2.70, plus 0.54 p.p.m. Cu^{++}) plotted as zero-order reaction (1); first-order reaction (2); and second-order reaction (3).

Some typical reactions are plotted (Fig. 1). The reactions are plotted as zero-, first-, and second-order, for comparison. A second-order law does not describe the reaction. In most cases the rate law of zero-order could be used to characterize the reaction adequately for the initial 50 per cent or so of its course. However, it appears that for the greatest portion of the reaction the first-order rate law is most descriptive and most generally valid. There are cases which do not seem to follow either law. These occur most notably above a pH of seven.

In one experiment the rate of oxidation of ascorbic acid in initial concentration varying from 10 to 100 mg. per cent in water was determined. Results of this experiment are shown (Table 2), where the rate constants are shown as first-order.

If suitable corrections are made for the pH of the solution, the specific reaction rate is reasonably constant throughout the range. Here it was assumed that the rate law followed is

$$(1) \qquad \frac{-d(H_2A)}{dt} = \frac{k(H_2A)}{(H^+)^{1/2}}$$

TABLE 2

Initial Conc. H_2A in mg. pct.	Initial pH	Final pH	K ($hr.^{-1}$)	Log K—1/2 pH
104.9	3.11	3.05	0.177	—2.31
91.3	3.02	.....	0.138	—2.37
86.4	3.28	3.25	0.184	—2.32
81.2	3.30	.....	0.161	—2.44
71.3	3.30	3.31	0.184	—2.38
71.0	3.33	.....	0.177	—2.41
66.2	3.31	3.33	0.253	—2.26
60.9	3.38	.....	0.209	—2.37
49.9	3.38	3.42	0.267	—2.26
30.8	3.52	3.62	0.444	—2.11
30.4	3.52	.....	0.391	—2.17
20.3	3.62	.....	0.483	—2.13
18.5	3.60	3.80	0.605	—2.13
10.1	3.82	.....	1.010	—1.91
10.0	3.80	4.00	1.060	—1.93

We have interpreted the pH as measured by the glass electrode to parallel the actual activity of hydrions as suggested by Bates (1948), and have assumed as a first approximation that the activity of ascorbic acid is roughly proportional to its concentration. The ionic strength of electrolytes was shown by Dekker and Dickinson (1940) to have little effect on the kinetics of the reaction and was neglected here and subsequently. The relative constancy of the expression (log K — 1/2 pH) supports these assumptions. This supports the conclusion that the rate is independent of the initial concentration of ascorbic acid and that the reaction is first-order.

Accordingly, all rate constants have been reported in this paper as first-order, specific rate constants. Where the data when plotted as log (H_2A) versus time does not give a straight line, the best line through the points exclusive of the initial point was taken. The slope of the log (H_2A) versus time curve multiplied by 2.3 is the specific rate constant.

Limiting Conditions of Oxygen Supply: To determine the rate of oxygen flow to be used, to assure an adequate amount of all determinations, a series of experiments was run in which flow of oxygen was varied. A particularly fast reaction was chosen as representing the extreme condition apt to be met. These results are shown (Table 3).

The data indicate that at any rate of oxygen flow above 56 ml. per minute there should be a sufficient supply for any reaction.

TABLE 3

Comparison of Varying Rates of Oxygen Flow on Rate of Oxidation of Ascorbic Acid in a Solution Buffered With 0.1 M H_3PO_4 + 0.1 M NaOH

Initial pH	O_2 rate ml./min.	Cu (p.p.m.)	Fe (p.p.m.)	K (hr^{-1})
7.06	0	0.02	0.07	0.51
7.07	20	0.02	0.07	1.10
7.06	25	0.02	0.07	3.91
7.09	56	0.02	0.07	5.22
7.07	100	0.02	0.07	5.22
7.07	150	0.02	0.07	5.22

Comparison of Rate of Auto-oxidation of Ascorbic Acid Isomers: A comparison of rate of oxidation of the different isomers of ascorbic acid was made. 1(—) ascorbic acid from the Eastern Company, with a specific rotation of —23.0 at 25°C., d-iso ascorbic acid from Hoffmann-La Roche, and the 1(±) ascorbic acid used in all other experiments were compared in buffered and unbuffered solutions. Results are shown (Table 4).

TABLE 4

Comparison of Rate of Auto-Oxidation of 1(—) Ascorbic Acid, d-iso Ascorbic Acid, and 1(±) Ascorbic Acid

pH	Type H_2A	K (hr^{-1})
In 0.1 M H_3PO_4 + 0.1 M NaOH buffered solutions		
3.10	1(—)	0.28
3.08	1(±)	0.42
3.08	d-iso	0.60
In unbuffered solutions		
3.48	1(—)	0.34
3.48	1(±)	0.45
3.48	d-iso	0.37

Results obtained indicate there are but slight differences between the three isomers under the conditions tested. The racemic mixture oxidizes at a more rapid rate in both buffered and unbuffered solutions, while the iso ascorbic acid oxidizes more rapidly in the buffered solution and less so in the unbuffered. Yourga, Esselen, and Fellers (1944) and Esselen, Powers, and Woodward (1945) reported that d-iso ascorbic acid oxidized more rapidly than l-ascorbic acid in a phosphate-citric acid buffered aqueous solution at pH four, and also found this to be true in food products. As will be reported later, we found this difference between l-ascorbic acid and d-iso ascorbic acid to be true also in the presence of 20 per cent dextrose.

Temperature Coefficient: Specific reaction constants were determined at temperatures of 35.8, 30, 25, and 20° in unbuffered solutions and in orthophosphate-buffered solutions at pHs of approximately three, five, and seven. Results of these measurements are shown (Table 5).

The Arrhenius constant calculated from the slope of log K versus reciprocal of absolute temperature for the unbuffered solutions at pH of 3.4 was 9,980; for phosphate buffers at pH 3.1, it was 11,250; for phosphate buffers at pH 4.6, 5,865; and for phosphate buffers at pH 7.1, 6,900. The average value of the Arrhenius constant was 8,500, neglecting the temperature coefficient for the phosphate buffer itself. In the phosphate buffers, the heat of activation includes not only the heat of activation of the oxygen-absorbing species, but also the heats of neutralization of ascorbic acid by phosphate and hydroxyl ions.

Average activation energy of ascorbic acid would correspond to about 16,900 and the temperature coefficient for this value of Q would be 2.52 in the range of 25 to 35°. This is higher than the temperature coefficient of 1.65 found by Barron *et al.* (1936) for the ratio of oxygen consumed in 30 minutes at 37 to 27° by a 1.0 M solution of ascorbic acid. The tem-

TABLE 5

Effect of Temperature on Auto-Oxidation of Ascorbic Acid

Temperature	Initial pH	Cu(p.p.m.)	Fe(p.p.m.)	K(hr^{-1})
In unbuffered solutions				
35.8	3.30	0	0	0.86
30.0	3.48	0	0	0.46
25.0	3.41	0	0	0.17
20.0	3.35	0	0	0.15
In 0.1 M H_3PO_4 + 0.1 M NaOH buffered solutions				
35.8	3.00	0.07	0.07	0.69
30.0	3.08	0.07	0.07	0.20
25.0	3.08	0.07	0.07	0.17
20.0	3.08	0.07	0.07	0.12
35.8	4.63	0.04	0.07	5.43
30.0	4.57	0.04	0.07	3.29
25.0	4.73	0.04	0.07	2.12
20.0	4.50	0.04	0.07	1.88
35.8	7.02	0.02	0.07	5.29
30.0	7.00	0.02	0.07	5.20
25.0	7.20	0.02	0.07	3.36
20.0	7.03	0.02	0.07	2.72

perature coefficients calculated only from the value of K at 35.8 and 25° for our data would be 5.05, 4.06, 2.56, and 1.57, respectively. These show a progressive decrease with increase in pH.

Effect of pH, Copper, and Iron: Barron *et al.* (1936), Dekker and Dickinson (1940), Silverblatt *et al.* (1943), Peterson and Walton (1943), and Weissberger *et al.* (1943, 1944) have investigated the effect of pH and copper concentration on auto-oxidation of ascorbic acid. Specific rate constants reported by the separate workers are not in agreement when compared under comparable condition of copper concentration, buffer, and pH. Most of these workers used Warburg apparatus or other condition of limited oxygen supply. It is generally agreed that copper is a powerful catalyst, that care must be taken to eliminate or control the numerous substances that complex with copper, and that the rate increases with increasing pH.

To obtain additional data on the effect of these factors on rate of reaction, a series of experiments was carried out in which pH, buffer, copper, and iron were varied in a controlled fashion so effects of each could be measured. Data obtained are summarized (Tables 6 to 9). In these tables (with the exception of Table 6, Series A) the measured pH value after mixing buffer and ascorbic acid is recorded, together with the actual Cu and Fe content of the solution. In Table 6 the copper and iron contents found were due to impurities in the buffers used, but in Tables 7, 8, and 9 the metal content was adjusted to various levels. During the course of oxidation the pH value changes somewhat. At pH values of 6.5 and below, the final pH value remains either essentially constant or increases by about 0.05 unit; above pH of 6.5, the pH decreases usually by 0.1 to 0.2 unit. In the latter cases products of oxidation other than dehydroascorbic acid must accumulate.

TABLE 6

Effect of Buffer on Rate of Oxidation of Ascorbic Acid

Initial pH	Cu (p.p.m.)	Fe (p.p.m.)	K (hr^{-1})
A. 0.2 M Na_2HPO_4 + 0.1 M citric acid solutions			
3	0.03	1.00	0.14
4	0.03	1.10	0.18
5	0.03	1.20	0.13
6	0.03	1.25	0.31
7	0.03	1.30	2.42
B. 0.1 M citric acid + 0.1 M NaOH			
3.09	0.04	0.36	0.13
4.02	0.03	0.25	0.11
4.97	0.02	0.19	0.10
5.87	0.02	0.15	0.17
6.59	0.02	0.14	0.20
C. 0.1 M H_3PO_4 +0.1 M NaOH			
3.00	0.23	0.01	0.67
3.70	0.22	0.01	1.03
4.45	0.22	0.01	>3.90
5.80	0.20	0.01	>3.90
6.70	0.17	0.01	>3.90
7.51	0.15	0.01	>3.90
D. 0.2 M HPO_3 + 0.1 M NaOH			
3.00	0.007	0.14	0.0027
3.87	0.007	0.13	0.0410
4.67	0.007	0.13	0.0800
5.80	0.005	0.12	0.0780
7.00	0.005	0.12	0.0460
7.70	0.004	0.08	0.1500
E. 0.1 M $H_2C_2O_4$ + 0.1 M NaOH			
3.08	0.03	0.28	0.0018
4.05	0.03	0.21	0.0053
5.10	0.02	0.19	0.0120
7.05	0.02	0.18	0.1380

TABLE 7

Effect of pH on Rate of Oxidation of Ascorbic Acid in 0.2 M Phosphate Buffer Adjusted to 0.50 p.p.m. of Cu^{++} and 10.0 p.p.m. of Fe^{+++}

pH	K (hr^{-1})	Log K—1/2 pH
2.10	1.01	—1.05
3.03	1.21	—1.44
3.90	7.20	—1.09
4.30	12.65	—1.15
5.65	17.80	—1.58
6.20	19.70	—1.80
6.52	25.60	—1.85
7.00	17.95	—2.25
7.35	13.50	—2.55

Data (Tables 6, 8, and 9) in general indicate a progressive increase in rate of oxidation, with increase in pH (except for Tables 6 and 8). These data indicate that the rate increases with pH up to about pH seven, then decreases, particularly at higher levels of copper content. That this is

TABLE 8

Effect of Copper on Rate of Oxidation of Ascorbic Acid

Initial pH	Cu (p.p.m.)	Fe (p.p.m.)	K (hr^{-1})
A. 0.1 M $H_2C_2O_4$ + 0.1 M NaOH solutions			
3.08	0.03	0.28	0.0018
4.05	0.03	0.21	0.0053
5.10	0.02	0.19	0.0120
7.05	0.02	0.18	0.1380
2.96	5.42	0.28	0.0011
3.96	5.42	0.21	0.0041
4.70	5.42	0.19	0.0160
9.38	5.42	0.18	17.8000
11.33	5.42	0.18	46.9000
B. 0.1 M H_3PO_4 + 0.1 M NaOH solutions			
3.00	0.23	0.01	0.67
3.70	0.22	0.01	1.03
2.70	5.42	.....	1.66
3.71	5.42	.....	17.20
5.80	5.42	.....	42.60
6.61	5.42	.....	62.60
8.40	5.42	.....	53.80
3.08	0.07	0.07	0.20
4.57	0.04	0.07	3.28
7.00	0.02	0.07	5.19
3.04	0.46	0.07	0.76
4.85	0.46	0.07	11.80
7.00	0.46	0.07	19.80
3.08	0.86	0.07	1.03
4.72	0.86	0.07	13.90
7.08	0.86	0.07	61.10

TABLE 9

Effect of Iron on Rate of Oxidation of Ascorbic Acid

Initial pH	Cu (p.p.m.)	Fe (p.p.m.)	K (hr^{-1})
In 0.1 M H_3PO_4 + 0.1 M NaOH solutions			
2.90	0.07	5.68	0.57
3.85	0.04	5.68	1.33
7.00	0.02	5.68	2.19
2.81	0.07	11.31	0.50
3.40	0.04	11.31	0.74
6.87	0.02	11.31	1.46
2.85	0.86	11.31	1.15
3.43	0.86	11.31	3.21
6.96	0.86	11.31	25.40

actually a real effect is borne out by data (Table 7), where the effect of pH was investigated in more detail. Existence of a maximum between pH six and seven is definitely indicated. The maxima and minima apparent in the pH-rate data for some of the other buffers in more acid regions are also real, since the results were verified by independent, duplicate experiments.

As can be seen (Table 6), the buffer used has a considerable effect on the rate. The rate in citrate-phosphate-buffered solutions differs by a 100-fold factor from that in oxalate-buffered solutions in which the copper content is roughly the same. The effect of the buffers must be explained by their different ability to complex copper. Oxalate has a great ability to reduce the catalyzing effect of copper, followed in lesser degrees by metaphosphate and citrate buffers. The orthophosphate buffers have the least power to reduce the catalytic effect of copper.

This difference between oxalate and orthophosphate buffers is brought out more strikingly (Table 8)—where the copper content of the two solutions has been adjusted to the same level. The difference in rate is essentially unchanged for the two levels of copper in the oxalate solutions, but is vastly different in the two orthophosphate solutions.

Effect of copper on the rate in the absence of complexing agents is brought out by the five levels of copper concentration tested in the orthophosphate-buffered solutions (Table 8-B).

Ability of iron (ferric) to catalyze the reaction is demonstrated (by data Table 9 and by comparing this data with that of Table 8). A much larger amount of ferric ion is necessary to catalyze the reaction and the effect is reversed at higher pH values. There appears to be an amount of ferric ion beyond which catalytic power is lost.

Dekker and Dickinson (1940) give the equation

$$(2) \qquad \frac{-d\ln(H_2A)}{dt} = \frac{k(Cu^{++})}{(H^+)^2}$$

to explain their results.

Silverblatt *et al.* (1943) give the equation

$$(3) \qquad k = \frac{K'(O_2)^0}{(H_2A)^{0.5}} \quad \frac{(CuSO_4)}{(H^+)^{0.7}}$$

to explain results they obtained.

Weissberger *et al.* (1943) reported that below pH seven the autoxidation is primarily that of the monovalent ascorbic acid ion,

$$\frac{-d(H_2A)}{dt} = k(HA^-)$$

and above pH seven it is that of the divalent ion. From the acid dissociation constants reported by Kumler and Daniels (1935) for ascorbic acid, $K_1 = 7.56 \times 10^{-5}$ ($pK_1 = 4.12$) and as $K_2 = 3.08 \times 10^{-12}$ ($pK_2 = 11.51$), they calculated the fraction of monovalent and divalent ions present in solutions at various pH values.

On the assumption that rate is governed by concentration of ascorbate ions (HA^-) and (Cu^{++}), Weissberger *et al.* (1944) proposed the following rate law

$$\frac{-d(H_2A)}{dt} = k\,(Cu^{++})(HA^-)$$

for pH values below seven. This rate law is followed fairly well in the region of pH 1.7 to 4, but is not followed above this pH, where auto-oxidation of the divalent ion becomes significant. Our data indicate that the mechanism of the oxidation of ascorbic acid is more complex than this rate law would indicate. Maxima occur at pH values below two and between six and seven which cannot be explained by the above assumption.

Data (Table 8) have been used to calculate the specific reaction constant k on the basis of equation (2). This equation gives constants which vary between the extremes in an amount of about 10^9. Apparently, the data cannot be explained on the basis of equation (2). In Table 10 the data of Table 8 have been compared using various assumptions for the over-all rate law. The necessary assumptions are apparent from headings of the columns.

TABLE 10

Comparison of Data on Basis of Various Assumptions for the Rate Law

Buffer—0.1 M H_3PO_4 + 0.1 M NaOH

pH	Cu^{++} p.p.m.	K (hr^{-1})	$K(H^+)^2 \times 10^6$	$K(H^+) \times 10^6$	$K(H^+)^{1/2} \times 10^6$	$\frac{K(H^+)^{1/2}}{(Cu^{++})} \times 10^4$	$\frac{K(H^+)^{1/2}}{(Cu^{++})^{1/2}} \times 10^4$
3.08	0.07	0.20	1.38×10^{-3}	16.60	578	825.0	218.0
4.57	0.04	3.28	.....	8.85	1,710	4,270.0	855.0
7.00	0.02	5.19	5.20×10^{-8}	0.05	165	825.0	117.0
3.04	0.46	0.76	6.30×10^{-2}	69.00	2,400	522.0	354.0
4.85	0.46	11.80	.....	16.80	4,440	964.0	655.0
7.00	0.46	19.80	1.98×10^{-6}	0.19	626	136.0	92.4
3.08	0.86	1.03	7.14×10^{-2}	86.60	2,990	348.0	321.0
4.72	0.86	13.90	.....	26.40	6,080	707.0	654.0
7.08	0.86	61.10	4.21×10^{-6}	0.51	1,760	205.0	189.0
2.70	5.42	1.66	6.67×10^{-1}	334.00	7,440	137.0	317.0
3.71	5.42	17.20	.....	336.00	24,200	446.0	1,030.0
5.80	5.42	42.60	.....	6.72	5,360	99.0	230.0
6.61	5.42	62.60	3.77×10^{-5}	1.53	3,110	57.4	133.0
3.00	0.23	0.67	.....	66.70	2,110	919.0	440.0
3.70	0.22	1.03	.....	20.30	1,430	650.0	304.0

The equation which best explains the data appears to be

$$(4) \qquad \frac{-d\log(H_2A)}{dt} = \frac{k(Cu^{++})^{1/2}}{2.3(H^+)^{0.5}}$$

Data from various authors were selected for comparison. Data used were chosen so that interference from complexing was not present and so that a wide range of pH values was present. This data is arranged (Table 11) and is calculated on the basis of a first-order reaction. The data is corrected to 30° by using the temperature coefficient found in our experiments.

TABLE 11

Comparison of Data From Various Authors (Corrected at 30°)

pH	Cu^{++} p.p.m.	Buffer	$K(hr^{-1})$	$K(H^+)10^6$	$\frac{K(H^+)10^6}{(Cu^{++})}$	$K(H^+)^{1/2}10^4$	$\frac{K(H^+)^{1/2}10^4}{(Cu^{++})}$	$\frac{K(H^+)^{1/2}10^4}{(Cu^{++})^{1/2}}$	Source[1]
1.67	32.00	Perchloric-acid	0.432	913.00	28.500	637.00	19.80	113.00	2
1.79	32.00	Perchloric-acid	0.750	1,210.00	37.800	954.00	29.80	168.00	2
1.95	32.00	Perchloric-acid	1.480	1,640.00	51.300	1,560.00	48.80	275.00	2
1.97	32.00	Perchloric-acid	1.160	1,230.00	38.400	1,200.00	37.40	212.00	2
1.97	6.41	Perchloric-acid	0.261	277.00	43.400	269.00	41.90	106.00	2
1.97	32.00	Perchloric-acid	1.340	1,420.00	44.300	1,380.00	43.10	244.00	2
1.97	6.41	Perchloric-acid	0.228	242.00	37.800	235.00	36.60	92.50	2
2.25	33.60	Perchloric-acid	3.970	2,210.00	65.900	2,970.00	88.50	513.00	2
2.59	1.27	Phosphate	0.004	1.03	0.810	2.03	1.60	1.80	5
3.00	1.00	Phosphate	1.330	133.00	133.000	420.00	420.00	420.00	4
3.17	6.40	Phosphate	1.310	88.50	13.800	340.000	53.20	134.00	1
3.42	0.13	Phosphate	0.950	36.00	277.000	185.00	1,430.00	514.00	3
3.42	0.64	Phosphate	2.600	99.00	15.400	508.00	795.00	635.00	3
3.87	1.27	Sodium acetate	0.040	0.54	0.425	4.65	3.66	4.13	1
4.05	6.40	Sodium acetate	1.850	16.50	2.570	175.00	27.40	69.20	1
5.10	6.40	Phosphate	2.970	2.36	0.370	84.00	13.10	33.20	1
5.62	1.27	Phosphate	0.170	0.041	0.030	2.64	2.08	2.34	5
6.00	6.40	Phosphate	4.450	0.445	0.069	44.50	6.95	17.60	1
6.05	1.00	Phosphate	30.300	2.700	2.700	285.00	285.00	285.00	4
6.20	1.00	Phosphate	33.900	2.140	2.140	268.00	268.00	268.00	4
6.66	1.27	Phosphate	0.280	0.006	0.005	1.31	1.03	1.16	5
6.95	6.40	Phosphate	6.500	0.023	0.011	21.80	3.41	8.62	1

[1] 1—Barron, de Meio, and Klemperer (1936); 2—Dekker and Dickinson (1940); 3—Silverblatt, Robinson, and King (1943); 4—Peterson and Walton (1943); 5—Weissberger, Lu Valle, and Thomas (1943, 1944), (uncorrected).

Data (Table 11) seem to fit equation (4) more closely than any of the other equations. The variation apparent in the equation (4) constants as calculated for these authors is from 1.16 to 635. Variations in our data are from 92.4 to 1,030. Average values are 191.7×10^{-4} and 394.0×10^{-4}, respectively.

SUMMARY

The kinetics of the oxidation of ascorbic acid by oxygen in the presence of copper and iron has been studied. It was found the reaction was essentially first-order with respect to ascorbic acid concentration, and its rate was directly proportional to the square root of the copper concentration and inversely as the square root of the hydrogen ion concentration in acid solutions. Ferric iron catalyzes the oxidation of ascorbic acid in the more acid solutions, but very slightly in comparison with copper. At pH values close to neutrality, ferric ions apparently inhibit the copper catalysis. The effect of iron and copper, however, was not studied separately; this would have entailed preparation of copper-free solutions in which the catalysis of iron might be studied in absence of any effect by copper. Oxidation of ascorbic acid is a reaction with a fairly high temperature coefficient, whose value is affected by pH and buffer. Type of buffer, as well as pH value, influences rate of oxidation; in buffers complexing copper the rate at the same pH level is markedly less. In order of decreasing complexing, the buffers are: oxalate, metaphosphate, citrate, and orthophosphate. There is evidence that maxima in rates of oxidation occur at low pH values and between pH six and seven. In view of this complex behavior, no mechanism for this oxidation has been given, particularly since the purpose of this study was to define the kinetics of oxidation of ascorbic acid for subsequent comparison of the effect of additions of various sugars.

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EFFECT OF SUGARS ON OXIDATION OF ASCORBIC ACID. II. GENERAL AND SPECIFIC EFFECTS[1]

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Several investigators have recently studied the effect of sugars on oxidation of ascorbic acid, under different conditions. with varying results—Munilla and Vogelsinger (1937), Chamrai (1941), Richardson and Mayfield (1944), and Sumuki, Yamanaka, Oka, and Takeishu (1944). With the exception of Chamrai (1941), no attempt was made to correlate results obtained with the possible effects of sugar in reducing oxygen solubility, decreasing the rate of diffusion of oxygen into solution, or copper-binding power. Chamrai (1941) suggested the protective effect of sucrose at low pH values was due to its effect in reducing rate of diffusion, and at high pH values to its copper binding power. His data, however, were limited to observations of loss in ascorbic acid content in unaerated solutions stored at room temperature. Investigations described in the present paper were carried out for the purpose of establishing the mechanism and extent of the protective effect of several pure sugars and of the commercial sugars and sirups.

MATERIALS AND PROCEDURE

Part I of this report contains all necessary data on materials used, with the exception of the sugars, Joslyn and Miller (1949). Essential characteristics of the sugars are shown (Table 1), including their iron and copper content, since these were found to have an important influence on the rate.

Solutions of sugar were prepared by dissolving 20 grams of sugar or sugar solids, for the 20 gram-per cent solution, or 40 grams for the 40 gram-per cent solution, in the desired buffer or water. If necesssary, heat was used to dissolve the sugar. The sugar solutions were then let stand, or cooled until room temperature was reached. Fifty mg. of ascorbic acid were weighed into a 100-ml. volumetric flask, the sugar solution and any necessary other solutions added, the whole diluted to volume, and the mixture shaken for from 20 to 30 seconds. At this point timing of the reaction was begun. A portion of the solution was then transferred to the reaction vessel, previously described, fitted with a porous plug-type bubbler, and placed in the bath maintained at 30°. Oxygen from a cylinder was bubbled through the solution at a rate of 100 ml. per minute throughout the reaction. The pH of the solution remaining was taken on a Beckman Industrial Model pH Meter within five minutes of the start of the reaction.

Samples were withdrawn from the reaction vessel at intervals and titrated for residual ascorbic acid by the method of Bessey and King, using 2,6-dichloroindophenol, as previously described.

[1] This work was started as part of a project supported by grants-in-aid from the Sugar Research Foundation.

TABLE 1[1]

Characteristics of the Sugars

No.	Sugar	Supplier	Grade	Lot	Dry basis Cu (p.p.m.)	Dry basis Fe(p.p.m.)	Solids
							pct.
1	Sucrose	Pfanstiehl	Reag.	853	0.000	5.00	100.0
2	Sucrose	Merck	Reag.	.74771	0.000	5.88	100.0
3	Dextrose	Merck	Reag.	.72623	0.335	.....	100.0
4	Dextrose	B & A	Reag.	35	0.000	5.05	100.0
5	Lactose	B & A	USP	15	0.700	14.40	100.0
6	Maltose	Pfanstiehl	CP	1694	0.200	11.20	100.0
7	Maltose	Difco	CP	Control 390274	0.100	8.00	100.0
8	Sweetose	Staley	Comm. sirup	.....	9.850	10.00	76.2
9	Sweetose	Staley	Comm. sirup	.....	1.980	15.40	84.2
10	Puritose	Corn Prods. Ref. Corp.	Comm. sirup	.....	1.120	28.10	84.0
11	Invert type 90	C & H Sugar Ref.	Sirup	.....	0.260	14.20	71.0
12	Invert type 0	C & H Sugar Ref.	Sirup	.....	0.380	15.20	66.5
13	Baker's glucose	Corn Prods. Ref. Corp.	Sirup	.....	0.700	8.30	84.3
14	Levulose	Eastman	CP	.....	0.100	7.60	100.0
15	Levulose	U. of Colo.	Tech.	Run 13	0.600	12.60	98.8

[1] Sugars 11 and 12 were samples of "liquid sugar," or heavy density sirups, prepared from cane sugar. Sugar 12 is a purified sucrose solution taken just before crystallizers, and Sugar 11 is the same sirup after inversion to the extent of 90 per cent. Sugars 10 and 13 are typical of high-conversion corn sirup and low-conversion corn sirup prepared by Corn Products Refining Corporation at Argo. Sugars 8 and 9 are samples of A. E. Staley's enzyme converted corn sirup; Sample 8 was from an early run in which enzymic action had continued until the sirup granulated as a result of precipitation of dextrose, and this sample had picked up excessive amounts of copper and iron; Sample 9 was obtained in 1946 and was more typical of the lots commercially available. Sugar 15 was from a pilot plant operation in which levulose was prepared from sucrose after inversion.

The sugars were analyzed for copper by the method of Coulson and Drabkin and for iron by the method of Saywell and Cunningham. Wet-ashing of the sugar with perchloric acid was used in conjunction with the iron analyses. No ashing was used for the copper analyses.

EXPERIMENTAL RESULTS AND DISCUSSION

Order of the Reaction: With sugars present in the reaction the problem of order of reaction is also present, as it was with solutions without sugars. Some typical data are plotted as zero-, first-, and second-order reactions (Fig. 1). Here again the first-order reaction seems best to describe the reaction. Of the 226 solutions tested in orthophosphate buffers in which the copper, iron, sugar concentrations, and sugar were varied, 48 or 21 per cent deviated markedly from a first-order rate law. Of these 28 were found in two of the sugars, sweetose and puritose. These two are converted corn sirups, and it may be there are impurities in the sirups which affect the reaction.

All reaction constants reported are first-order constants and were obtained by multiplying the slope of the log (concentration) versus time (in hours) plot by 2.3. Where marked deviation from a straight line occurred, the best line through the points, exclusive of the first point, was used.

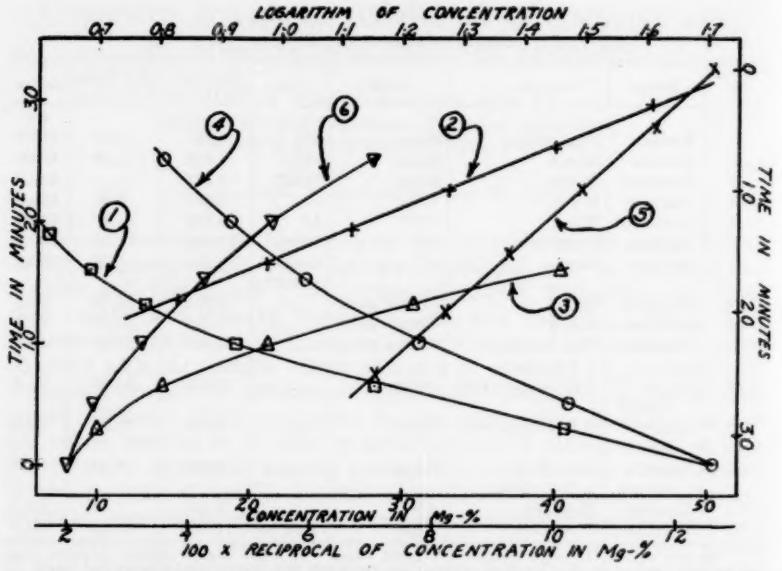
LOGARITHM OF CONCENTRATION
0.7 0.8 0.9 1.0 1.1 1.2 1.3 1.4 1.5 1.6 1.7
TIME IN MINUTES
30 20 10 0
0 10 20 30
1 2 3 4 5 6
CONCENTRATION IN Mg-%
10 20 30 40 50
2 4 6 8 10 12
100 x RECIPROCAL OF CONCENTRATION IN Mg-%

FIG. 1. Typical data for auto-oxidation of ascorbic acid in presence of sugar (40 gram-per cent levulose in phosphate buffer).

1. pH 4.80 plotted as zero-order reaction.
2. pH 4.80 plotted as first-order reaction.
3. pH 4.80 plotted as second-order reaction.
4. pH 6.52 plotted as zero-order reaction.
5. pH 6.52 plotted as first-order reaction.
6. pH 6.52 plotted as second-order reaction.

Effect of Amount of Oxygen: Water solutions of the sugars were made in 20 and 40 gram-per cent. After addition of ascorbic acid these solutions were allowed to stand at room temperature in 100-ml. beakers covered with watch glasses. At 24-hour intervals the solutions were adjusted for evaporation by adding water and the residual ascorbic acid determined by titration. Solutions of sugar and water and ascorbic acid made in a similar fashion were placed in the bath, with oxygen bubbled through at a rate of 100 ml. per minute. These solutions were titrated at suitable intervals for residual ascorbic acid. In this way a comparison is afforded for solutions with a surplus of oxygen and for those with a limited supply. Results of these experiments are shown (Table 2).

The unaerated solutions were held at approximately 24°. Comparison of these sets of data shows at once the strong dependence of rate of reaction on availability of oxygen. Under the conditions of limited oxygen supply all sugars tested reduced the extent of the initial oxidation, with the exception of a sample of sweetose with unusually high copper content. The protective effect was less with lactose and greater with levulose. Doubling the concentration of sugar decreased the amount of oxidation, even though it doubled the copper and iron content of the solutions, since the sugars used were the chief sources of these metals. In the latter case the sugars, in order of decreasing oxidation, were sweetose, sucrose, baker's

TABLE 2

Comparison of Effect of Sugars in Presence and Absence of Sufficient Oxygen

Sugar	Cu (p.p.m.)	Fe (p.p.m.)	pH (20% soln.)	pH (40% soln.)	20 per cent solution Per cent oxidized				40 per cent solution Per cent oxidized			
					Standing		O_2 at 100 c.c./min.		Standing		O_2 at 100 c.c./min.	
					24 hr.	48 hr.	1 hr.	2 hr.	24 hr.	48 hr.	1 hr.	2 hr.
Sucrose	0.00	5.0	2.8	2.4	47.3	74.6	36.3	61.1	20.60	30.0	16.80	24.7
Levulose	0.10	7.6	3.0	2.8	28.0	55.3	47.4	69.0	12.20	17.8	17.40	32.2
Maltose	0.20	11.2	3.5	3.9	39.1	54.9	71.9	97.8	7.97	10.0	7.15	12.6
Type 90	0.26	14.2	3.0	.....	32.0	55.9	43.3	74.1	18.20	21.2	36.20	61.6
Dextrose	0.33	.....	2.4	2.5	37.9	64.9	23.5	45.1	12.70	21.9	9.07	14.9
Type 0	0.38	15.2	3.2	3.4	44.9	66.5	45.2	83.3	20.90	36.5	23.70	48.0
Lactose	0.70	14.4	2.6	2.7	51.1	88.8	60.7	84.5	17.10	30.4	41.60	62.9
Baker's glucose	0.70	8.3	2.9	3.0	37.9	58.3	60.4	88.2	18.80	30.3	24.40	50.5
Puritose	1.12	28.1	2.6	2.9	33.8	47.1	60.8	81.5	9.60	15.7	10.70	25.0
Sweetose	9.85	10.0	.....	3.9	93.0	100.0	97.6	100.0	25.50	48.3	98.50	100.0
Water	.....	.....	2.8	.....	53.1	80.5	38.5	62.0	.....	.....	.....	.....

glucose, type 0 sirup, type 90 sirup, lactose, dextrose, levulose, and maltose. In the same solutions, markedly different results were observed when oxygen was bubbled through them. In this case, at the lower level of sugar content only dextrose and levulose exerted a protective effect, initially. Here, too, doubling the concentration of sugar decreased the extent of oxidation except in the case of sweetose. Apparently here the copper content was the limiting factor. In the order of decreasing oxidation the sugars were sweetose, lactose, type 90, baker's glucose, type 0 levulose, sucrose, puritose, dextrose, and maltose. The variations in cupric and ferric content, and in pH, however, were such as to make it impossible to evaluate the specific effect of the sugars. These data are typical of those reported in the literature in which control of pH, Cu, and Fe was not exercised.

Twenty gram-per cent sweetose in orthophosphate solution was oxidized at 30°, with varying rates of oxygen flow, to test if the flow of 100 ml. per minute was adequate to assure an excess of oxygen for a moderately rapid reaction. Most of our measured rates were below a K of six. Results of this experiment are shown (Table 3).

TABLE 3

Effect of Oxygen on Rate of Auto-oxidation of Ascorbic Acid

20 gram-per cent sweetose (Sugar No. 9) in 0.1 M H_3PO_4 + 0.1 M NaOH

pH	O_2 rate ml./min.	Cu (p.p.m.)	Fe (p.p.m.)	K (Hr^{-1})
4.42	150	0.44	3.20	6.10
4.43	100	0.44	3.20	6.10
4.43	56	0.44	3.20	6.10
4.42	25	0.44	3.20	0.84
4.42	0	0.44	3.20	0.62

Evidently a rate of O_2 flow above 56 ml. per minute is sufficient for relatively rapid oxidations.

Temperature Coefficient: The temperature coefficient of the auto-oxidation of ascorbic acid under conditions of vigorous oxidation was determined for dextrose (Sugar No. 4) in orthophosphate-buffered solutions. Temperatures used were 20, 25, 30, and 35.8°. These results are shown (Table 4).

From these data the value of the Arrhenius constant was calculated at the four pH levels and found to be 10,419 at pH 3.32; 11,431 at pH 2.96; 12,075 at pH 4,35; and 7,613 at pH 6.84. The average value—10,380—is appreciably higher than the 8,500 found for the orthophosphate solutions without sugar.

Comparison of Rate of Auto-Oxidation of l-Ascorbic Acid, l(±)-Ascorbic Acid, and d-iso Ascorbic Acid: l-Ascorbic acid from the Eastman Company with a specific rotation of —23.0 at 25°, d-iso ascorbic acid from Hoffmann-La Roche Company, and l(±)-ascorbic acid from Hoffmann-La Roche Company were compared in 20 gram-per cent solutions of dextrose (Sugar No. 4), in water, and in ortho-phosphate-buffered solutions. Results are shown (Table 5).

TABLE 4

Effect of Temperature on Rate of Oxidation

20 gram-per cent dextrose (Sugar No. 4) in 0.1 M H_3PO_4 + 0.1 M NaOH

pH	Temperature	Cu (p.p.m.)	Fe (p.p.m.)	K(Hr^{-1})
3.28	35.8	0.00	1.17	0.977
3.33	30.0	0.00	1.17	0.292
3.32	25.0	0.00	1.17	0.170
3.33	20.0	0.00	1.17	0.145
2.92	35.8	0.07	1.24	0.725
2.98	30.0	0.07	1.24	0.384
2.95	25.0	0.07	1.24	0.131
3.00	20.0	0.07	1.24	0.096
4.27	35.8	0.04	1.24	7.620
4.29	25.0	0.04	1.24	1.490
4.50	20.0	0.04	1.24	0.910
6.82	35.8	0.02	1.24	8.250
6.93	25.0	0.02	1.24	3.200
6.78	20.0	0.02	1.24	2.260

In the presence of sugars the d-iso ascorbic acid is oxidized at a more rapid rate than the l isomers. The racemic mixture is oxidized at a slightly lower rate than the optically active l isomer.

Effect of Cupric Ions, Ferric Ions, pH, and Sugar Concentration on Rate of Auto-Oxidation of Ascorbic Acid: The problem of discovering the effect of pH, copper, and iron and sugars on the rate of auto-oxidation of ascorbic acid was first attacked by studying the rate in variously buffered solutions. It was hoped that differences in sugars could thus be quantitatively measured in terms of the copper-binding power of some of the buffers. Data obtained from this study are shown (Table 6), in which the effect of 20 gram-per cent of sugars was studied in five different buffers.

TABLE 5

Comparison of Rate of Auto-oxidation of l-Ascorbic Acid, l(±)-Ascorbic Acid, and d-Iso Ascorbic Acid in 20 Gram-Per Cent Dextrose Solutions

pH	Type H_2A	K(Hr^{-1})
In water		
3.36	l	0.338
3.33	l(±)	0.315
3.33	d-iso	0.510
In 0.1 M H_3PO_4 + 0.1 M NaOH		
2.98	l	0.400
2.98	l(±)	0.362
2.98	d-iso	1.080

Rate constants obtained with sucrose in the citrate-phosphate buffer were somewhat higher at the lower pH values, but were definitely lower than those found for this buffer at the same copper level in absence of sugar at pH levels of five and above. In the citrate buffer sucrose exerted but little effect at all pH values; in the metaphosphate buffer the rate in presence of sucrose was somewhat lower, particularly at pH seven, than

TABLE 6

Rate of Auto-oxidation of Ascorbic Acid in Various Sugars and in Variously Buffered Solutions

Part 1
20 gram-per cent sucrose (Sugar No. 1)

pH	Cu (p.p.m.)	Fe (p.p.m.)	K (Hr^{-1})
In 0.2 M Na_2HPO_4 + 0.1 M Citric Acid			
3.00	0.03	2.45	0.161
4.00	0.03	2.30	0.207
5.00	0.03	2.25	0.101
6.00	0.03	2.20	0.237
7.00	0.03	2.10	1.570
In 0.2 M HPO_3 + 0.1 M NaOH			
3.00	0.01	1.08	0.0014
3.80	0.01	1.12	0.0220
4.48	0.01	1.12	0.0690
5.65	0.01	1.13	0.0410
7.00	0.01	1.13	0.0510
7.50	0.01	1.14	0.0870
In 0.1 M $H_2C_2O_4$ + 0.1 M NaOH			
3.00	0.03	1.28	0.0029
4.00	0.03	1.21	0.0034
4.98	0.02	1.19	0.0110
5.98	0.02	1.18	0.0690
In 0.1 M H_3PO_4 + 0.1 M NaOH			
2.90	0.23	1.01	0.306
3.60	0.22	1.01	0.783
4.08	0.22	1.01	2.340
In 0.1 M Citric + 0.1 M NaOH			
3.07	0.04	1.36	0.115
4.00	0.03	1.25	0.213
4.90	0.02	1.19	0.118
5.81	0.02	1.15	0.111
6.51	0.02	1.14	0.180

Part 2
20 gram-per cent dextrose (Sugar No. 3)

pH	Cu (p.p.m.)	Fe (p.p.m.)	K (Hr^{-1})
In 0.2 M Na_2HPO_4 + 0.1 M NaOH			
2.98	0.10	.....	0.161
4.00	0.10	.....	0.207
5.00	0.10	.....	0.104
6.00	0.10	.....	0.237
7.00	0.10	.....	1.520
In 0.2 M HPO_3 + 0.1 M NaOH			
2.97	0.07	.....	0.0028
3.76	0.07	.....	0.0240
4.48	0.07	.....	0.0770
5.61	0.07	.....	0.0480
7.00	0.07	.....	0.0440
7.45	0.07	.....	0.1150
In 0.1 M $H_2C_2O_4$ + 0.1 M NaOH			
2.98	0.10	.....	0.0037
3.97	0.10	.....	0.0062
4.93	0.10	.....	0.0120
6.72	0.10	.....	0.1600
In 0.1 M H_3PO_4 + 0.1 M NaOH			
2.88	0.30	.....	0.280
3.60	0.29	.....	0.782
4.22	0.29	.....	3.220
In 0.1 M Citric Acid + 0.1 M NaOH			
3.02	0.11	.....	0.143
3.98	0.10	.....	0.215
4.89	0.09	.....	0.147
5.81	0.09	.....	0.154
6.44	0.09	.....	0.177

Part 3
20 gram-per cent lactose (Sugar No. 5)

pH	Cu (p.p.m.)	Fe (p.p.m.)	K (Hr^{-1})
In 0.2 M Na_2HPO_4 + 0.1 M Citric Acid			
4.00	0.17	3.98	1.020
5.00	0.17	4.08	0.600
6.00	0.17	4.13	0.932
7.00	0.17	4.18	3.180
In 0.1 M HPO_3 + 0.1 M NaOH			
3.00	0.15	3.02	0.012
3.68	0.15	3.01	0.051
4.65	0.15	3.01	0.186
5.69	0.15	3.00	0.136
7.00	0.14	3.00	0.117
7.42	0.14	2.96	0.186
In 0.1 M $H_2C_2O_4$ + 0.1 M NaOH			
2.96	0.17	3.16	0.0026
4.05	0.17	3.09	0.0075
4.98	0.16	3.07	0.0053
6.35	0.16	3.06	0.3380
In 0.1 M H_3PO_4 + 0.1 M NaOH			
3.08	0.37	2.89	0.690
3.60	0.36	2.89	1.570
In 0.1 M Citric Acid + 0.1 M NaOH			
3.08	0.18	2.24	0.414
4.01	0.17	2.13	1.140
4.92	0.16	2.07	0.775
5.80	0.16	2.03	0.681
6.52	0.16	2.02	0.598

Part 4
20 gram-per cent maltose (Sugar No. 6)

pH	Cu (p.p.m.)	Fe (p.p.m.)	K (Hr^{-1})
In 0.2 M Na_2HPO_4 + 0.1 M NaOH			
4.0	0.07	3.34	0.955
5.0	0.07	3.54	0.414
6.0	0.07	3.49	0.850
7.0	0.07	3.54	0.685
7.7	0.07	3.69	0.300
In 0.1 M HPO_3 + 0.1 M NaOH			
3.10	0.41	2.36	0.032
3.78	0.41	2.35	0.129
4.49	0.41	2.35	0.117
5.65	0.41	2.34	0.140
7.00	0.41	2.34	0.113
7.40	0.40	2.30	0.184
In 0.1 M $H_2C_2O_4$ + 0.1 M NaOH			
3.00	0.07	2.52	0.0033
4.05	0.07	2.45	0.0140
4.92	0.06	2.43	0.0850
6.20	0.06	2.42	0.4250
In 0.1 M H_3PO_4 + 0.1 M NaOH			
3.08	0.27	2.25	0.250
3.75	0.26	2.25	0.878
4.28	0.26	2.25	1.150
5.40	0.24	2.25	2.390
6.59	0.21	2.25	1.010
7.23	0.19	2.25	0.630
In 0.1 M Citric Acid + 0.1 M NaOH			
3.08	0.08	2.70	0.183
4.00	0.07	2.49	0.755
4.90	0.06	2.43	0.910
5.78	0.06	2.39	0.665
6.50	0.06	2.38	0.505

TABLE 6 *(Concluded)*

Rate of Auto-oxidation of Ascorbic Acid in Various Sugars and in Variously Buffered Solutions

Part 5

20 gram-per cent sweetose (Sugar No. 8)

pH	Cu (p.p.m.)	Fe (p.p.m.)	K (Hr^{-1})	pH	Cu (p.p.m.)	Fe (p.p.m.)	K (Hr^{-1})
In 0.2 M Na_2HPO_4 + 0.1 M NaOH				In 0.1 M $H_2C_2O_4$ + 0.1 M NaOH			
4.00	2.00	3.10	2.260	3.05	2.00	2.28	0.0039
5.00	2.00	3.20	2.530	4.02	2.00	2.21	0.0140
6.00	2.00	3.25	3.180	4.67	1.99	2.19	0.0320
				5.85	1.99	2.18	0.2070
In 0.1 M HPO_3 + 0.1 M NaOH				In 0.1 M H_3PO_4 + 0.1 M NaOH			
3.22	1.98	2.14	0.258	3.21	2.20	3.01	3.500
3.91	1.98	2.13	1.680	In 0.1 M Citric Acid + 0.1 H NaOH			
4.50	1.98	2.13	2.710	3.10	2.01	2.36	1.080
5.39	1.97	2.12	1.940	3.99	2.00	2.25	2.710
7.00	1.97	2.12	1.200	5.69	1.99	2.15	2.120
7.10	1.97	2.08	1.210	6.32	1.99	2.14	2.230

in its absence; in the oxalate buffer the rate was affected markedly at pH six only, at which it was less than in the absence of sugar.

Comparison for the other sugars tested could not be made because of the higher copper and iron contents of the buffers containing added sugars. In the case of sucrose at about the same pH value, rate of oxidation was least in metaphosphate buffer, then in oxalate, citrate, and orthophosphate, respectively, in order of increasing rate. Rate of oxidation in the presence of sucrose was roughly about 10 to 100 times as rapid in orthophosphate as in metaphosphate. Similar buffer effects were observed in dextrose, lactose, and sweetose solutions, in spite of the higher copper and iron levels encountered. In the case of dextrose, however, the metaphosphate was only slightly superior to oxalate in suppressing oxidation; in the case of lactose, oxalate was definitely superior to metaphosphate, and this was true of maltose and sweetose, as well. Metaphosphate and oxalate apparently are more effective in copper-binding power than the sugars used. While the data indicate that differences do exist in ability of sugars to suppress oxidation of ascorbic acid, differences in copper and iron content render it impossible to determine the relative specific effect of sugar. Attempts to calculate an equation for the ratio observed were unsuccessful. The variation in copper-binding power of sugars with pH apparently is involved.

Relative specific effects of the sugars can be compared, however, at the same level of copper, iron, and hydrogen ion concentration. One phase of this study was completed. For this work orthophosphate-buffered solutions were chosen as representing the least amount of interference with cupric and ferric ions. Solutions were made in which the cupric ion, the ferric ion, and the ferric and cupric concentrations varied together. These were run at pH values of three, five, and seven, and at sugar concentrations of 20 and 40 gram-per cent. Rate constants were obtained in this way on seven sugars. Data obtained are given (Table 7).

Data (Table 7) definitely indicate that sugars do inhibit oxidation of ascorbic acid in a pH range above 4.5. Extent of inhibition increases with increase in concentration of sugar. Some sugars are more effective inhibi-

TABLE 7

Rate Constants for Auto-oxidation of Ascorbic Acid in Presence of Sugar and With Adjusted Copper and Iron Concentration

A					B				
Type 90 solutions (Sugar No. 11)					Dextrose solutions (Sugar No. 4)				
pH	Sugar conc.	Cu (p.p.m.)	Fe (p.p.m.)	K(Hr^{-1})	pH	Sugar conc.	Cu (p.p.m.)	Fe (p.p.m.)	K(Hr^{-1})
2.98	20	0.12	5.68	0.563	2.70	20	0.07	5.68	0.370
4.13	20	0.09	5.68	1.830	3.85	20	0.04	5.68	1.390
6.82	20	0.07	5.68	3.230	6.80	20	0.02	5.68	2.580
2.78	20	0.12	11.31	0.395	2.68	20	0.07	11.31	0.481
4.20	20	0.09	11.31	2.200	5.61	20	0.04	11.31	4.430
6.63	20	0.07	11.31	3.230	6.65	20	0.02	11.31	1.920
3.07	20	0.47	2.90	0.865	2.94	20	0.47	1.24	0.745
4.41	20	0.47	2.90	9.750	4.40	20	0.47	1.24	8.830
6.85	20	0.47	2.90	17.300	6.80	20	0.47	1.24	17.300
3.08	20	0.86	2.90	1.230	2.94	20	0.86	1.24	0.895
4.60	20	0.86	2.90	14.300	4.43	20	0.86	1.24	10.500
6.82	20	0.86	2.90	24.200	6.88	20	0.86	1.24	20.200
2.76	20	0.86	11.31	1.020	2.89	20	0.86	11.31	1.270
4.20	20	0.86	11.31	11.100	4.05	20	0.86	11.31	8.350
6.80	20	0.86	11.31	16.300	6.77	20	0.86	11.31	16.800
2.90	40	0.18	11.31	0.186	3.25	40	0.00	2.41	0.197
4.60	40	0.15	11.31	1.000	2.58	40	0.07	11.31	0.285
6.51	40	0.13	11.31	1.410	4.45	40	0.04	11.31	3.330
2.52	40	0.18	22.62	0.364	6.39	40	0.02	11.31	2.610
4.32	40	0.15	22.62	1.310	2.35	40	0.07	22.62	0.610
6.00	40	0.13	22.62	0.966	3.18	40	0.04	22.62	0.356
3.18	40	0.86	5.66	1.330	5.68	40	0.02	22.62	2.100
4.39	40	0.86	5.66	7.450	2.84	40	0.86	2.41	0.773
6.55	40	0.86	5.66	12.700	4.18	40	0.86	2.41	6.590
3.17	40	1.65	5.66	4.140	6.60	40	0.86	2.41	12.300
4.42	40	1.65	5.66	13.300	2.84	40	1.65	2.41	1.030
6.52	40	1.65	5.66	18.100	4.20	40	1.65	2.41	8.000
3.10	40	1.65	22.62	4.330	6.58	40	1.65	2.41	15.900
4.62	40	1.65	22.62	11.600	2.28	40	1.65	22.62	0.875
6.30	40	1.65	22.62	11.900	4.90	40	1.65	22.62	9.110
					6.42	40	1.65	22.62	9.050

TABLE 7 *(Continued)*

Rate Constants for Auto-oxidation of Ascorbic Acid in Presence of Sugar and With Adjusted Copper and Iron Concentration

C					D				
Sucrose solutions (Sugar No. 2)					Maltose solutions (Sugar No. 7)				
pH	Sugar conc.	Cu (p.p.m.)	Fe (p.p.m.)	K(Hr^{-1})	pH	Sugar conc.	Cu (p.p.m.)	Fe (p.p.m.)	K(Hr^{-1})
2.85	20	0.07	5.68	0.632	3.07	20	0.17	1.60	0.579
3.84	20	0.04	5.68	1.610	4.54	20	0.14	1.60	3.550
6.52	20	0.02	5.68	1.930	6.83	20	0.12	1.60	1.480
2.72	20	0.07	11.31	0.790	3.02	20	0.17	5.68	0.618
3.32	20	0.04	11.31	0.564	4.48	20	0.14	5.68	4.300
6.68	20	0.02	11.31	1.640	6.81	20	0.12	5.68	1.440
3.00	20	0.47	1.08	0.735	3.00	20	0.17	11.31	0.623
3.00	20	0.47	1.08	0.690	4.30	20	0.14	11.31	3.560
3.38	20	0.47	0.07	0.735	6.79	20	0.12	11.31	1.230
4.55	20	0.47	1.08	8.390	3.04	20	0.47	1.67	0.775
6.89	20	0.47	1.08	17.500	4.53	20	0.47	1.67	8.700
4.50	20	0.86	1.08	11.800	6.88	20	0.47	1.67	8.640
6.92	20	0.86	1.08	25.900	3.06	20	0.86	1.67	1.150
2.92	20	0.86	11.31	1.620	4.49	20	0.86	1.67	11.700
3.94	20	0.86	11.31	10.400	6.85	20	0.86	1.67	12.900
6.83	20	0.86	11.31	14.800	3.00	20	0.86	11.31	1.300
3.19	40	0.00	2.02	0.165	4.22	20	0.86	11.31	9.450
2.68	40	0.07	11.31	0.489	6.80	20	0.86	11.31	9.900
3.28	40	0.04	11.31	0.341	3.08	40	0.27	3.20	0.364
6.80	40	0.02	11.31	0.797	4.41	40	0.24	3.20	1.640
2.50	40	0.07	22.62	0.683	6.71	40	0.22	3.20	1.260
3.28	40	0.04	22.62	0.477	3.01	40	0.27	11.31	0.425
6.18	40	0.02	22.62	0.917	4.20	40	0.24	11.31	1.900
2.95	40	0.86	2.09	0.860	6.67	40	0.22	11.31	0.980
4.28	40	0.86	2.09	8.340	2.94	40	0.27	22.62	0.425
6.75	40	0.86	2.09	14.300	3.85	40	0.24	22.62	1.250
2.90	40	1.65	2.09	1.320	6.55	40	0.22	22.62	0.945
4.30	40	1.65	2.09	9.000	3.08	40	0.86	3.27	0.945
6.73	40	1.65	2.09	21.600	4.39	40	0.86	3.27	8.070
2.35	40	1.65	22.62	1.220	6.73	40	0.86	3.27	6.380
4.95	40	1.65	22.62	9.900	3.06	40	1.65	3.27	1.630
6.50	40	1.65	22.62	8.730	4.32	40	1.65	3.27	10.700
					6.72	40	1.65	3.27	11.600
					2.90	40	1.65	22.62	1.630
					3.78	40	1.65	22.62	6.200
					6.56	40	1.65	22.62	8.970

TABLE 7 *(Continued)*

Rate Constants for Auto-oxidation of Ascorbic Acid in Presence of Sugar and With Adjusted Copper and Iron Concentration

E					F				
Sweetose solutions (Sugar No. 9)					Puritose solutions (Sugar No. 10)				
pH	Sugar conc.	Cu (p.p.m.)	Fe (p.p.m.)	K(Hr^{-1})	pH	Sugar conc.	Cu (p.p.m.)	Fe (p.p.m.)	K(Hr^{-1})
3.13	20	0.47	3.20	0.995	3.04	20	0.30	5.68	0.512
4.60	20	0.47	3.20	2.920	3.80	20	0.27	5.68	0.475
6.82	20	0.47	3.20	3.130	6.88	20	0.25	5.68	0.261
3.02	20	0.47	5.68	1.130	2.85	20	0.30	11.31	0.527
4.22	20	0.44	5.68	4.600	3.70	20	0.27	11.31	0.504
6.80	20	0.42	5.68	2.830	6.76	20	0.25	11.31	0.436
2.87	20	0.47	11.31	1.000	3.02	20	0.47	5.68	0.835
3.66	20	0.44	11.31	2.560	4.51	20	0.47	5.68	2.520
6.67	20	0.42	11.31	3.180	6.80	20	0.47	5.68	1.470
3.13	20	0.86	3.13	1.400	3.02	20	0.86	5.68	1.380
4.59	20	0.86	3.13	9.200	4.05	20	0.86	5.68	6.500
6.80	20	0.86	3.13	5.350	6.82	20	0.86	5.68	8.000
3.82	20	0.79	3.13	2.790	2.84	20	0.86	11.31	1.270
3.81	20	1.97	3.13	6.530	4.02	20	0.86	11.31	8.630
3.10	20	0.86	11.31	1.890	6.80	20	0.86	11.31	6.020
4.16	20	0.86	11.31	10.000	3.00	40	0.51	11.31	0.140
6.70	20	0.86	11.31	5.700	4.20	40	0.48	11.31	0.197
3.35	40	0.86	6.33	0.935	6.61	40	0.46	11.31	0.184
4.32	40	0.86	6.33	2.800	2.72	40	0.51	22.62	0.351
6.49	40	0.86	6.33	2.450	4.05	40	0.48	22.62	0.368
3.08	40	0.86	11.31	0.945	6.23	40	0.46	22.62	0.257
4.62	40	0.83	11.31	3.870	3.07	40	0.86	11.31	0.700
6.40	40	0.81	11.31	2.970	4.20	40	0.86	11.31	2.610
2.72	40	0.86	22.62	1.350	6.50	40	0.86	11.31	1.290
4.30	40	0.83	22.62	3.520	3.08	40	1.65	11.31	3.980
5.88	40	0.81	22.62	2.170	4.20	40	1.65	11.31	7.460
3.95	40	0.79	6.26	3.170	6.52	40	1.65	11.31	6.150
4.00	40	1.58	6.26	5.300	3.01	40	1.65	22.62	3.180
3.35	40	1.65	6.33	2.740	5.18	40	1.65	22.62	6.370
4.38	40	1.65	6.33	7.250	6.42	40	1.65	22.62	4.690
6.48	40	1.65	6.33	4.120					
3.28	40	1.65	22.62	3.500					
4.85	40	1.65	22.62	7.910					
6.30	40	1.65	22.62	4.840					

TABLE 7 *(Concluded)*

Rate Constants for Auto-oxidation of Ascorbic Acid in Presence of Sugar and With Adjusted Copper and Iron Concentration

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Levulose solutions (Sugar No. 15)

pH	Sugar conc.	Cu (p.p.m.)	Fe (p.p.m.)	K(Hr^{-1})	pH	Sugar conc.	Cu (p.p.m.)	Fe (p.p.m.)	K(Hr^{-1})
2.91	20	0.19	5.68	0.603	4.29	40	0.28	11.31	0.157
5.00	20	0.16	5.68	0.345	6.35	40	0.26	11.31	0.510
6.70	20	0.14	5.68	0.333	2.51	40	0.31	22.62	0.790
2.74	20	0.19	11.31	0.959	3.08	40	0.28	22.62	0.088
4.08	20	0.16	11.31	0.119	5.95	40	0.26	22.62	0.368
6.55	20	0.14	11.31	0.663	3.49	40	0.24	5.04	0.040
2.99	20	0.47	2.59	0.932	2.97	30	0.86	5.11	0.306
4.43	20	0.47	2.59	0.061	4.21	40	0.86	5.11	0.023
6.91	20	0.47	2.59	0.208	6.70	40	0.86	5.11	0.167
3.00	20	0.86	2.59	0.972	2.97	40	1.65	5.11	0.863
4.56	20	0.86	2.59	7.940	4.19	40	1.65	5.11	6.500
6.88	20	0.86	2.59	5.480	6.72	40	1.65	5.11	5.220
2.95	20	0.86	11.31	1.190	2.88	40	1.65	22.62	1.220
4.15	20	0.86	11.31	6.400	4.80	40	1.65	22.62	6.740
6.78	20	0.86	11.31	3.060	6.52	40	1.65	22.62	3.100
2.78	40	0.31	11.31	0.264					

tors than others. Cupric ion is a powerful catalyst for this oxidation, and its catalytic effect is exerted over the entire range of pH studied. Ferric ion, however, is a less effective catalyst, and its catalytic effect extends only to a pH of about 3.5 to four; at pH levels above this it is an inhibitor. The mechanism of protection by sugars is probably by formation of cupric-sugar complexes, but the sugars tested, in concentrations up to 40 per cent, suppress the catalytic power of only an amount of copper equivalent to about one p.p.m. This effect is more evident by direct comparison of sugars at about one p.p.m. of copper.

Rate constants of the reaction, in the presence of sugars and 0.86 p.p.m. cupric ion and 11.31 p.p.m. ferric ion, have been compared at three pH levels to rate constants at these levels in the absence of sugars. The differences have been expressed as per cent inhibition by dividing the difference of the two by the rate constant without sugar and multiplying by 100. Rate constants at exactly pH three, 4.5, and seven have been chosen by interpolating the smooth curve drawn from the data. Results of this comparison appear (Table 8).

On the basis of this comparison the sugars can be ranked in effectiveness from least to greatest as type 0, dextrose, sucrose, maltose, sweetose, puritose, and levulose. It is obvious the protective effect of sugars is less at pH three than at pH 4.5 or seven, apparently due to the greater ability of sugars to complex copper ions at higher pH value. If this is the only effect of sugars under these conditions, then the levulose-copper complex must be less dissociated in acid solutions than those of other sugars. Factors other than copper ion binding must be involved, but these cannot be evaluated from the present data. Because the effect of sugars varies so much with pH, it is difficult to find a rate law that will apply to the data available.

TABLE 8

Comparison of Effectiveness of Sugars in Inhibiting Auto-oxidation of Ascorbic Acid in Presence of 0.86 p.p.m. Copper and 11.31 p.p.m. Iron

pH	3	4.5	7	3	4.5	7
Per cent sugar		20			40	
Sugar—Type 90—(Sugar No. 11) Per cent inhibition	0.0	0.0	36.1	0.0	26.0	64.4
Sugar—Dextrose—(Sugar No. 4) Per cent inhibition	0.0	1.9	37.7	0.0	25.0	73.7
Sugar—Sucrose—(Sugar No. 2) Per cent inhibition	0.0	0.0	43.0	0.0	11.5	70.8
Sugar—Maltose—(Sugar No. 7) Per cent inhibition	14.0	9.1	62.4	5.5	22.1	89.0
Sugar—Sweetose—(Sugar No. 9) Per cent inhibition	14.0	0.0	81.8	53.6	61.5	89.5
Sugar—Puritose—(Sugar No. 10) Per cent inhibition	0.0	17.3	74.5	66.7	74.0	98.9
Sugar—Levulose—(Sugar No. 15) Per cent inhibition	0.0	36.8	89.7	67.3	99.5	99.5

There are little data available on the chemistry of the copper complexes of sugar, but the ability of polyhydroxy compounds to form complexes, particularly in alkaline solutions, is well recognized, Bates *et al.* (1942), and Bobtelsky and Jordan (1945). The relative effectiveness of the sugars and of ascorbic acid to bind cupric ions as molecular-coordinated compounds is not known. If coordination complexes are formed with the anions, then the relative degrees of dissociation of the sugars will be a factor. Michaelis and Rona (1913) reported the acid dissociation constants of sugars at 18°C.(64°F.) to be glucose—6.6×10^{-13}; fructose—9.0×10^{-13}; sucrose—2.4×10^{-12}; maltose—1.8×10^{-12}; lactose—6.1×10^{-13}; galactose—5.2×10^{-13}; and mannose—1.1×10^{-12}. On the bases of these data it would be expected that in order of their ability to form copper salts the sugars could be arranged as follows: sucrose, maltose, fructose, glucose; but this is not the order of their effectiveness in suppressing oxidation of ascorbic acid.

SUMMARY

Rate of oxidation of ascorbic acid, under conditions of limited oxygen concentration and under conditions where oxygen concentration was not limiting, was determined in various buffers and at various pH levels, in the presence of varying amounts of cupric and ferric ions and in the presence of two levels of concentration of several sugars. Under the conditions of limited oxygen supply, all sugars tested reduced the initial rate of oxidation, with the exception of a sample of sweetose with excessively high copper content. The protective effect was less with lactose and greater with levulose. In the same solutions markedly different effects were observed when sufficient oxygen was bubbled through. Here dextrose was initially more protective. The large variations in cupric and ferric content of these solutions, however, made it difficult to draw any specific conclu-

sions. These data are typical of those already reported in the literature. When these investigations were extended to solutions adjusted to the same levels of copper and iron content, and at the same pH values in orthophosphate buffers, markedly different conditions were observed.

Under these conditions, the extent of protection by the sugars increased with increase in concentration of sugar and increase in pH. Sugars tested were ranked in order of decreasing protection as levulose, puritose, sweetose, maltose, sucrose, dextrose, and type 0 sirup. No simple rate law was found to govern oxidation of ascorbic acid in presence of sugars. The reaction was essentially first-order with respect to ascorbic acid. Copper catalyzed the rate over all pH range tested, but iron was catalytic only in the more acid range up to a pH of about four, and inhibitive beyond this range. The temperature coefficient of the oxidation in presence of sugars was similar to that found for ascorbic acid in sugar-free solutions.

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EFFECT OF SUGARS ON OXIDATION OF ASCORBIC ACID. III. INFLUENCE OF pH AND TYPE OF BUFFER

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The general course of oxidation of ascorbic acid in the presence of sugars has been indicated in previous papers in this series. To investigate the effect of pH and concentration of sugars in more detail, the following investigations were carried out.

MATERIALS AND PROCEDURE

Materials and procedure for the vigorously oxidized solutions were the same as those used for Parts I and II of this series, except as noted below, Joslyn and Miller (1949) and Miller and Joslyn (1949).

Solutions: Orthophosphoric acid and sodium hydroxide were used exclusively as the buffers in this part of the experiments. These buffers were made by adding solid sodium hydroxide to a 0.1 M solution of orthophosphoric acid until the desired pH was obtained. Thus, all buffered solutions had the same initial concentration of orthophosphoric acid. The solutions were analyzed for copper and iron, as previously noted.

Sugars: Sugars used are described (Table 1).

TABLE 1

Characteristics of the Sugars

No.	Sugar	Supplier	Grade	Lot	Cu (p.p.m.)	Fe (p.p.m.)
1	Sucrose	C & H Sugar Ref.	Comm.	.....	0.25	7.5
2	Dextrose	Merck	Reag.	42867	0.45	7.5
3	Levulose	U. of Colorado	Tech.	.....	.....[1]	.....[1]
4	Sucrose	C & H Sugar Ref.	Comm.	.....	0.17	4.5

[1] See record below of treatment of levulose to remove copper and iron.

The levulose was a second 10-pound lot received from the University of Colorado. This lot contained 2.2 p.p.m. copper and 33.2 p.p.m. iron. Since this was too high a concentration of metals, means had to be found to reduce the copper and iron concentration. Recrystallization of the levulose from alcohol and water did not reduce the concentration of the undesirable metals. The following treatment of levulose to reduce the concentration of metals was then used:

A solution of distilled water and sugar in 40-per cent concentration was made. To this solution, potassium ferrocyanide solution in the ratio of 0.0375 mM of potassium ferrocyanide to 50 grams of sugar was added. The mixture was allowed to stand for two days with 1.87 ml. of five per cent bentonite per 50 grams of sugar, then filtered through a fritted glass funnel with added supercel. Absence of any blue color, when ferric nitrate solution was poured into a small portion of the filtrate, was taken

as assurance that no excess ferrocyanide ions remained in the solution. An analysis for copper and iron was performed on the filtrate and it was found that the metals were in low enough concentration to permit use of the sugar in the experiments.

A portion of the levulose treated in this way was set aside for preparation of the 10-, 20-, and 30-per cent solutions. This portion had 0.10 p.p.m. copper and 12.8 p.p.m. iron based on the dried sugar. The remainder was concentrated in a vacuum oven operating at 55° and 29 inches' vacuum. Portions were drawn off at 52.9, 56.9, and 74.9 per cent for use in the 40-, 50-, and 60-per cent levulose solutions. The time in the vacuum oven was 72 hours for the lowest concentration and 170 hours for the highest. These portions each had 0.15 p.p.m. copper and 13.4 p.p.m. iron based on the dried sugar. The analysis for levulose in the solution was taken by refractive index measurements and the per cent levulose calculated from the table given in Circular C440 of the National Bureau of Standards, Bates and Associates (1942).

In order to control the preparation of solutions more accurately, particularly with regard to the actual concentration of ions present, all sugar solutions were made from a table of specific gravities and percentages. Values used are reproduced (Table 2).

TABLE 2

Basis for Preparation of Sugar Solutions

Sugar	Specific gravity	Sugar	Added solvent
pct.		*gm./100 ml. sol.*	*gm./100 ml. sol.*
10	1.038	10.4	93.4
20	1.080	21.6	86.4
30	1.127	33.8	79.8
40	1.176	47.1	70.5
50	1.230	61.5	61.5
60	1.286	77.2	51.4

Copper and iron were added to the solutions on the basis of 0.50 p.p.m. cupric ion and 10 p.p.m. ferric ion. To keep the added metallic ions on a comparable basis in each solution, and to maintain the ratio of catalyzing ions to ascorbic acid, each ion was added on the basis of an assumed weight of 100 grams for the final solutions. Since in each case 50 mg. of ascorbic acid were added for each 100 ml. of solution, this meant that the same initial concentration of copper and iron with respect to ascorbic acid was present.

Procedure: Preparation of sugar solutions for analysis under vigorous oxygenation was accomplished by dissolving the required amount of sugar in buffer, using heat if necessary. The solution was then placed in the 30° bath in a 100-ml. volumetric flask until temperature equilibrium was reached; then as quickly as possible (usually about three minutes) 50 mg. of ascorbic acid and the calculated amount of cupric nitrate and ferric nitrate solutions were added. The solution was made to volume, shaken vigorously for 20 seconds, timing of the reaction started, approximately 75 ml. poured into the reaction vessel, the vessel fitted with the porous

plug bubbler, and oxygen from a cylinder passed through at a rate of 100 ml. per minute throughout the course of the reaction.

In the case of the levulose, where the sugar was already in solution due to the treatment with potassium ferrocyanide, the properly buffered pH was secured by adding the calculated amount of orthophosphoric acid in 40 grams per 100 ml. concentration and sodium hydroxide in 25 grams per 100 ml. concentration. The remainder of the treatment was the same.

Solutions in which oxygen was not passed through, or the standing solutions, as they will be called here, were prepared in the same fashion, except that these solutions were not previously heated to 30° before start of the analysis. In addition, the full 100 ml. of solution was poured into the reaction vessel, since it was found that size of the sample had an effect on rate of oxidation. The reaction vessel for these standing solutions was a straight-sided glass vessel of 950-ml. capacity fitted with a ground-glass stopper. Dimensions of this bottle were 17 cm. in height by 8.3 cm. in diameter. The bottle was submerged to its neck in the 30° bath. During the course of the reaction, except for brief periods for sample removal, the vessel was kept stoppered.

Analysis of the solutions for residual ascorbic acid and pH was as described in previous papers.

EXPERIMENTAL RESULTS AND DISCUSSION

All results of these experiments are reported as first-order reaction constants, even though the reaction does not follow this law strictly throughout its course.

Effect of pH on Rate of Reaction: The change in the rate of reaction with pH has been discussed at some length in the first series of these papers. Due to the expanded range of hydrogen ion concentration used in this series of experiments, and to the slightly different concentrations of the buffer used, the effect of pH was more thoroughly investigated. In particular, the two concentrations of buffer used were compared at the same concentrations of cupric and ferric ions, at pH values of 1.6 to 7.3. Values of the reaction constant plotted versus pH are shown (Fig. 1).

It is apparent that at hydrogen ion concentrations below 10^{-6} M rate of the reaction is in some way dependent on concentration of phosphate present. At higher hydrogen ion concentrations, where there is an increase in rate of reaction, which is contrary to the observed rate law, the effect could be explained as an increase in the catalyzing power of ferric ions, as was observed in the earlier experiments. These effects need further study.

Effect of Sugar Concentration on Rate of Reaction: Three sugars were selected for more extensive investigation. These sugars were prepared in 10-, 20-, 30-, 40-, 50-, and 60-per cent concentrations in orthophosphoric acid-sodium hydroxide buffered solutions. The pH range studied was from 1.5 to 7.5. In each solution the cupric ion concentration was adjusted to 0.50 p.p.m. by the addition of cupric nitrate solution and the ferric ion concentration adjusted to 10 p.p.m. by addition of ferric nitrate solution. Thus, all sugars could be compared at the same concentration of copper and iron. Summarized data for sucrose is contained (Fig. 2); for dextrose (Fig. 3); and levulose (Fig. 4).

Due to the fact that even the auto-oxidation of ascorbic acid in the absence of sugars is not completely explained, as witness the behavior at both ends of the pH reaction rate data (Fig. 1), an attempt will not be made to derive equations for results found from the study of sugar solutions. Results shown in the figures are self-explanatory and show at once that at all concentrations and at all pH values rate of oxidation is less in solutions of sugar than in solutions in which sugar is absent. Depression

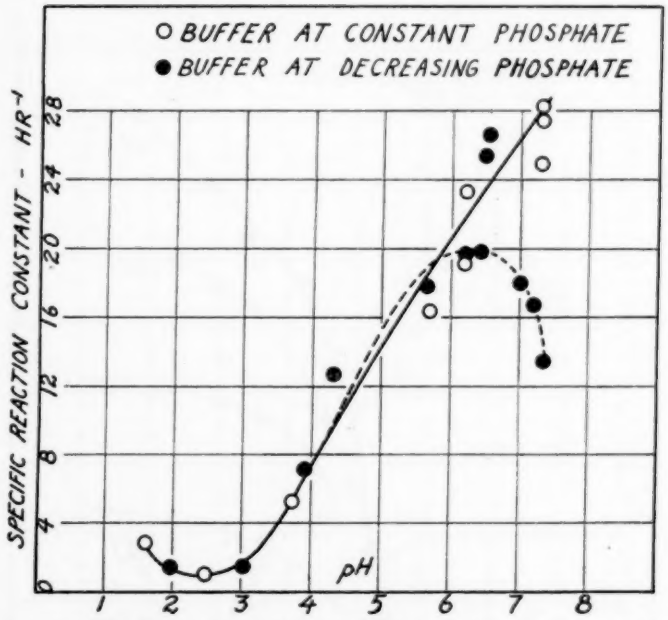

FIG. 1. Rate of oxidation of ascorbic acid in ortho-phosphoric acid-sodium hydroxide buffers in presence of 0.5 p.p.m. of Cu^{++} and 10 p.p.m. of Fe^{+++}.

The buffer at constant phosphate was prepared by adding 1.67, 2.91, 4.17, 4.50, 5.50, and 7.91 gm. of NaOH, respectively, to 11.53 gm. of 85 per cent phosphoric acid, and diluting to 1,000 ml. to prepare buffers of pH 2.0, 3.0, 4.3, 5.7, 6.3, and 7.3, respectively. The buffer at variable phosphate concentration was prepared by mixing 521, 493, 483, 454, 378, and 345 ml. of 0.1 M phosphoric acid, respectively, with 479, 507, 517, 546, 622, and 625 ml. of 0.1 M sodium hydroxide, respectively, for buffers at pH 3.0, 3.7, 4.5, 5.8, 6.7, and 7.5, respectively.

of the rate is greatest at higher pH values and increases uniformly with concentration of sugar. There is an increase in rate below a pH of 2.5, which is not readily explained but which was apparent in all solutions studied at these concentrations of hydrogen ions.

Levulose used in these experiments, treated as it was to reduce the copper and iron concentration, does not compare in rate of reaction under comparable conditions with levulose used in the experiment described in Part II of this paper. When the discrepancy was noticed, some of the origi-

nal lot of levulose was compared directly with the sugar that was ferrocyanide-treated. Under the same conditions of copper, iron, and sugar concentrations, and indeed at the same time, these two sugars showed vastly different effects on rate of oxidation of ascorbic acid. The ascorbic acid in the ferrocyanide-treated sugar was 50 per cent oxidized in 8.3 minutes; whereas, in the untreated levulose (original sample) the ascorbic acid was 50 per cent oxidized in 130 minutes. This large difference must remain unexplained until a complete analysis can be made of the two sugars for possible contaminants or decomposition products.

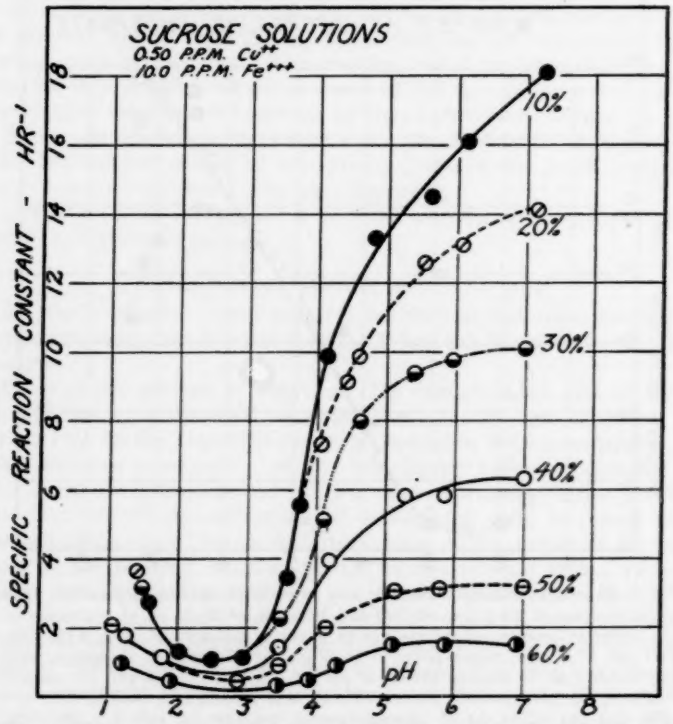

FIG. 2. Rate of oxidation of ascorbic acid in presence of various concentrations of sucrose in ortho-phosphoric acid-sodium hydroxide buffers.

Differences between the sugars are shown (Table 3), in which rate of reaction at four selected pH levels is computed as a per cent of the reaction constant at that pH for the buffer without added sugar.

There are only minor differences in the effect these sugars have on rate of oxidation of ascorbic acid.

Rate of Reaction in Standing Solutions: Rate of auto-oxidation of ascorbic acid in the presence of sugar and in the absence of sufficient oxygen was studied. For this purpose a large closed vessel was selected

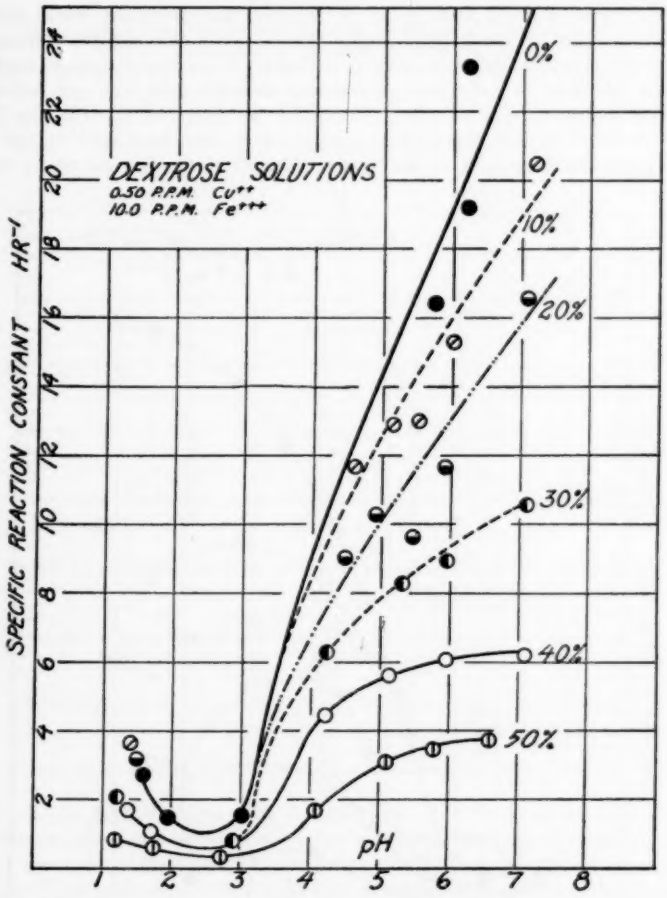

FIG. 3. Rate of oxidation of ascorbic acid in presence of various concentrations of dextrose in ortho-phosphoric acid-sodium hydroxide buffers.

which would contain sufficient oxygen in its air space above the liquid sample to constitute a suitably large excess over that needed to completely oxidize the ascorbic acid present. In addition, it was desired to hold to a low value the change in concentration due to evaporation, so that corrections for this error need not be made. A 950-ml. capacity, ground-glass, stoppered, glass reaction vessel was selected. All standing solutions were run in these vessels, in the 30° bath, with the flasks stoppered.

Preliminary experiments, in which a 250-ml. sample was used with 0.50 p.p.m. cupric ion, 10 p.p.m. ferric ion, and 50 mg. per cent ascorbic acid, did not give reproducible results. The major portion of the reaction

occurred between eight and 16 hours' reaction and therefore could not be studied. It was subsequently found that size of the sample influenced rate of the reaction, due probably to diffusion of oxygen from the surface into the solution. A difference in residual ascorbic acid was not found at different depths of the solution. To speed the reaction, so that the bulk of it occurred before eight hours, sample size was decreased to 100 ml. and cupric ion increased to one p.p.m. Reactions carried on under these

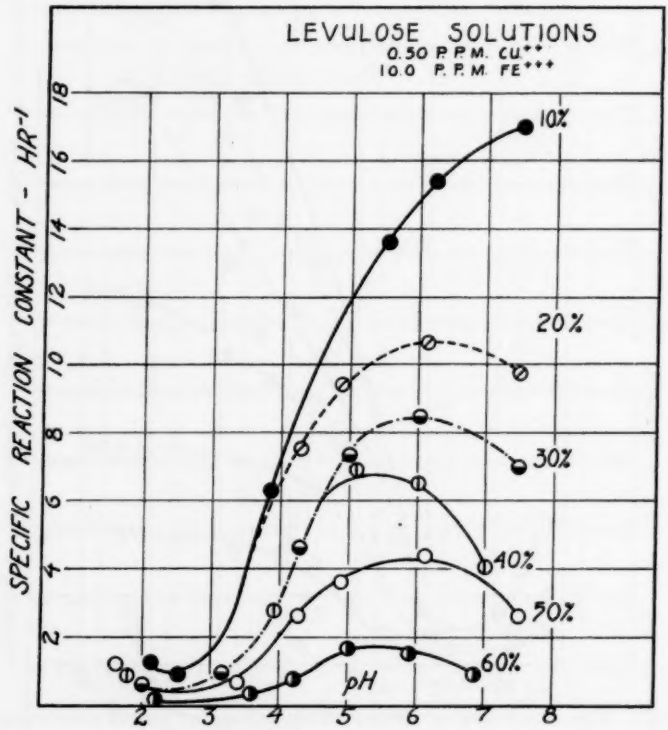

FIG. 4. Rate of oxidation of ascorbic acid in presence of various concentrations of levulose in ortho-phosphoric acid-sodium hydroxide buffers.

conditions were successfully studied within an eight-hour period. All experiments were then performed under these conditions.

In each of the solutions studied it was always observed that an initial rapid disappearance of ascorbic acid took place. Such rapid disappearance took place in from one to two hours, and usually amounted to from 10 to 15 mg. of ascorbic acid. Following this initial rapid oxidation, the remainder of the ascorbic acid was consumed at a much slower pace. The initial oxidation could be assumed to be the reaction between dissolved oxygen and ascorbic acid, and the subsequent disappearance due to reac-

TABLE 3

Comparison of Rate of Reaction of Sugars at Certain pHs With Rate of Reaction Without Sugars

Sucrose				
pH	1.5	2.5	4.5	6.5
Sugar	Reaction rate without sugar			
pct.	*pct.*	*pct.*	*pct.*	*pct.*
10	91.2	100	105.0	71.8
20	64.6	95	82.2	58.8
30	58.8	80	67.2	42.9
40	44.1	72	39.8	27.0
50	32.2	55	22.1	13.7
60	22.0	27	9.7	6.4
Dextrose				
pH	1.5	2.5	4.5	6.5
Sugar	Reaction rate without sugar			
pct.	*pct.*	*pct.*	*pct.*	*pct.*
10	58.90	90	93.9	74.2
20	51.40	80	73.4	61.8
30	42.60	60	60.6	41.8
40	35.30	50	42.9	26.2
50	2.15	40	20.8	16.1
Levulose				
pH	1.5	2.5	4.5	6.5
Sugar	Reaction rate without sugar			
pct.	*pct.*	*pct.*	*pct.*	*pct.*
10	....	90	85.4	67.8
20	....	68	74.8	45.9
30	....	54	50.4	35.1
40	....	54	48.7	24.9
50	....	42	27.4	17.6
60	....	10	09.3	5.1

tion with oxygen diffused into the solution. A brown color developed in solutions with a pH above four after standing for longer than 18 hours. The brown color increased in intensity with pH and with initial concentration of ascorbic acid. This color was assumed to be polymerized ascorbic acid.

Plotting the logarithm of residual ascorbic acid versus time did not give uniformly good curves in all cases, nor did a plot of residual ascorbic acid versus time. The data do not seem to warrant assumption of either a zero- or a first-order reaction. Data are reported (Table 4) as one-half life values, since this seemed to be all the accuracy the data would warrant. One-half life values for buffer, sucrose, dextrose, and levulose, in the concentrations indicated, are given (Table 4). Sugars are the same as used for the oxygenated solutions.

As can be seen from data (Table 4), rate of reaction when insufficient oxygen is present decreases steadily with increasing concentration of sugar. There is a maximum rate of reaction in the neighborhood of pH five. The rate at pH values above five appears to be complex.

TABLE 4

One-Half Life of Standing Solutions

SUCROSE

Per cent sugar	0		0		10		20	
	pH	hr.	pH	hr.	pH	hr.	pH	hr.
	2.10	6.84	2.11	7.30	2.15	13.60	2.01	19.00
	3.03	4.46	3.03	4.75	3.02	10.00	3.00	12.40
	3.71	3.20	3.75	3.55	3.79	5.94	3.68	7.28
	5.67	1.73	5.70	2.04	5.60	3.58	5.50	4.23
	6.22	1.38	6.23	1.76	6.12	3.67	6.01	4.67
	7.30	2.13	7.30	1.93	7.20	4.03	7.18	5.35
Per cent sugar	30		40		50		60	
	pH	hr.	pH	hr.	pH	hr.	pH	hr.
	2.01	23.10	1.93	44.1	1.93	62.5	1.91	95.0
	3.00	18.60	2.90	26.2	2.91	41.2	2.88	100.0
	3.63	10.30	3.60	18.9	3.60	30.4	3.59	68.0
	5.39	6.92	5.25	13.8	5.16	22.9	.....	.....
	5.92	7.19	5.79	17.0	6.09	28.4	5.60	78.5
	7.02	7.64	6.93	15.5	6.61	25.6	6.82	117.5

DEXTROSE

Per cent sugar	10		20		30		40		50	
	pH	hr.	pH	hr.	pH	hr.	pH	hr.	pH	hr.
	1.92	16.60	1.95	17.70	1.80	24.90	2.23	49.8	1.77	70.8
	3.00	10.80	2.97	13.20	2.85	18.70	.....	.....	2.79	57.3
	3.74	6.94	3.69	10.00	3.51	15.20	3.50	25.9	3.49	43.5
	5.68	3.50	5.50	5.52	5.30	9.42	5.18	16.4	5.10	28.3
	6.25	4.00	6.08	7.00	5.91	13.80	6.36	21.9	5.65	35.8
	7.40	4.12	7.19	6.69	7.00	12.80	6.60	20.6	6.75	32.8

LEVULOSE

Per cent sugar	10		20		30		40		50	
	pH	hr.	pH	hr.	pH	hr.	pH	hr.	pH	hr.
	2.02	15.00	2.01	17.60	2.10	27.30	2.08	47.10	2.52	68.00
	2.45	13.20	2.74	15.40	3.02	28.50	2.90	43.10	.....	.....
	3.94	5.78	3.59	12.20	3.63	16.10	3.50	27.20	4.30	32.30
	5.38	4.27	5.30	7.94	4.98	11.90	4.50	17.60	4.93	38.50
	6.20	5.31	6.01	9.40	5.86	14.10	6.11	20.50	5.95	29.30
	7.30	4.61	7.02	7.00	7.15	11.80	7.82	18.90	8.52	39.60

SUMMARY AND CONCLUSIONS

Rate of oxidation of ascorbic acid in phosphate buffers at pH 1.5 to 7.5 and in presence of levulose, dextrose, and sucrose at concentrations ranging from 10 to 60 per cent, was investigated in solutions supplied with excess oxygen and in solutions containing limited amounts of oxygen. In the absence of sugars reaction rate decreased from pH 1.5 to a minimum of pH 2.5, then increased with pH up to 7.5 when the total phosphate concentration was maintained constant. When the phosphate concentration decreased with increase in pH, the rate reached a maximum at pH 6.5, then decreased. The effect of phosphate in maintaining the

rate of increase with pH in the range of pH 6 to 7.5 was unexpected and cannot be explained on the basis of the available data. The sugars used depressed the rate of oxidation at all pH levels, but particularly so at higher pH levels. Sucrose and dextrose behaved similarly, but inhibition with levulose was more marked at pH above 5.5. In unoxygenated solutions the half-life period of ascorbic acid increased with increase in sugar concentrations, particularly at pH above five.

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BACTERIOLOGY OF SHRIMP.[1] I. INTRODUCTION AND DEVELOPMENT OF EXPERIMENTAL PROCEDURES

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According to the First Biennial Report of the Department of Wild Life and Fisheries, State of Louisiana, this state produced 75 per cent of the total shrimp production of the United States and Alaska in 1944 and 1945. Over 500,000 barrels, or 105,000,000 pounds, were obtained from Louisiana coastal waters in each of these years. Fishery Resources of the United States, Senate Document No. 51 (1945), reported that shrimp were the most valuable fishery resource on our South Atlantic and Gulf Coasts and supported a larger industry than any other fishery of those regions. In 1943, $15 million worth of shrimp were caught. Of this amount 46 per cent were sold fresh, 25 per cent canned, 15 per cent frozen, 10 per cent dried, and four per cent were marketed as cooked and peeled shrimp. Since 1943, the percentage marketed as frozen shrimp has greatly increased.

A number of workers have pointed out that shrimp are a perishable food requiring adequate refrigeration and expeditious handling, Clarke (1937), Clark and MacNaughton (1917), and Cameron and Williams (1934, 1934a). Better refrigeration methods needed in handling and marketing shrimp were emphasized by DuBois (1946), who stated that the ideal situation would result from installing freezing equipment on board the trawler. Conditions affecting the quality of frozen shrimp have been pointed out by Fieger and DuBois (1946), who concluded that highest quality product was obtained when the shrimp were prepared and frozen immediately after being taken from the nets.

The present study was undertaken to obtain and record bacteriological data on shrimp from the time caught until marketed as a fresh or frozen product. It was considered necessary to analyze the commercial methods of catching, storing, and marketing shrimp, in order to determine the cause of spoilage losses. Bacteriological methods applicable on fishing vessels, for determining total counts, coliform counts, and types of bacteria present were investigated. Various problems concerned with sampling, mixing, and plating shrimp tissues were studied and a standardized method for bacterial plate counts established.

Shrimp examined in these investigations were caught in outside Louisiana waters, i.e., the Gulf of Mexico. Fishing was limited to the area west of the mouth of the Mississippi River and east of the Sabine River. The distance from shore varied from eight to 28 miles, and the depth of the water from two to 20 fathoms (12 to 120 feet).

Methods of Handling and Storing Shrimp on Board Trawlers: The usual trawler for shrimping in outside waters is a boat at least 50 feet

[1] This project was supported in part by a grant from The Refrigeration Research Foundation, Berkeley, California.

long, carrying a three-man crew and sufficient ice for 10 days' fishing. Shrimp are caught in an otter trawl which is pulled along the Gulf bottom for from one to two hours. When the trawl is pulled in, the bag is raised and the catch dropped through the bottom of the net onto the deck. Usually 30 to 80 per cent of the catch is shrimp and the remainder small fish. Most of the shrimp caught in the Gulf of Mexico are large, the so-called Jumbos, averaging about 40 grams each. Immediately after dumping a net, the fishermen begin separating shrimp from fish, using small wooden rakes to spread the catch in a thin layer on the deck. During the first few days of a trip all shrimp are "headed" before icing, since by experience fishermen have found that headless iced shrimp have a longer storage life than whole shrimp. If the percentage of shrimp is large, heading and separation from fish are accomplished in one operation. No unusual care is exercised to prevent crushing or injuring the shrimp flesh. Experienced fishermen head shrimp without leaving parts of the "head" (cephalothorax) attached, but all heading methods involve tearing or twisting the head from the "tail" by hand. Some fishermen wear cotton gloves while handling the catch.

The washing procedure varies with the size of the catch and the individual preferences of the captain. Wire bushel baskets are filled two-thirds to three-quarters full and shaken from side to side, while a strong stream of sea water from a hose about four inches in diameter is forced through the shrimp for from one to two minutes. If the catch is unusually large, all shrimp are placed in the large cone-shaped wash-net and pulled through the sea for about five minutes. On a few boats, the shrimp are placed in a hand-net and dipped in barrels of sea water.

After washing and draining, the shrimp are iced in bins, in the hold of the boat. Headless and whole shrimp are stored in separate bins. Crushed or shaved ice stored in the hold is placed in a six-inch layer on the floor of a bin, and alternate layers of shrimp and ice are held in place by wooden boards at the front of the bin. Each day crushed ice is added at the edges and front of the bin, but the shrimp are not otherwise disturbed unless excessive melting occurs. Extensive melting necessitates re-icing the shrimp in another bin. A temperature of 0°C.(32°F.) is reached within shrimp flesh after one day's storage in ice. A well-insulated hold will keep shrimp adequately iced in the bins for approximately nine days, during hottest weather.

Speed is essential in all operations dealing with shrimp, both because they spoil quickly on the warm deck and because a new catch may be pulled in within the next two hours. The time between dumping a net and icing the catch does not usually exceed two hours, unless the catch is very large or delays due to weather or machinery-breakdown occur.

Fishermen regard nine to 10 days a maximum time for holding iced headless shrimp aboard trawlers. Whole shrimp are usually held not longer than four or five days before sale to a purchasing house. Here the bins are opened and shrimp and ice are shoveled into baskets which are emptied into a washing tank. From the tank the shrimp are picked up by a conveyer and carried to a work-table where the whole shrimp are headed. All shrimp are washed in a second tank before distribution to markets. If they are to be marketed as fresh shrimp, they are packed with ice in wooden

crates and refrigerated until trucked to dealers. Some reach consumer channels as frozen or canned shrimp, but a discussion of these methods is beyond the scope of this report.

Bacteriological Equipment and Preparations: All bacteriological work on freshly caught [2] and iced shrimp was done aboard the fishing vessels. The amount and kind of materials were limited by the bacteriological work to be done and dependent on the storage space, working space, and equipment available on the trawler. The most desirable working space on one boat was a six- by six-foot top deck cabin; on another trawler the galley, with its stationary table and benches, was the best available space despite its use for the preparation of meals. A Diesel or gasoline cooking stove was available on all trips and was used for the small pressure cooker in which materials were sterilized. The stove oven served for hot-air sterilization.

In so far as possible, materials were prepared and sterilized before boarding the boat. Culture media (in six-ounce medicine bottles and test tubes), water blanks (pyrex A.P.H.A. milk dilution bottles and test tubes), and mixing containers (Erlenmeyer flasks or Waring Blendor bowls) were sterilized in an autoclave at 121°C.(250°F.) for from 15 to 30 minutes. Adequate wrapping or protection was provided to insure sterility during transportation and storage. Petri dishes, wrapped in newspaper, pipettes in copper cans, squares of weighing paper, and instruments such as forceps, scissors, and needles were sterilized in hot-air oven at 170°C.(338°F.) for two hours. The Waring Blendor bowls and the dilution water used in these were sterilized as needed in the pressure cooker on the trawler.

Boro-silicate blendor bowls of 500-ml. capacity were used for mixing the samples. In the latter portion of this work, 1,000-ml. bowls were available, and permitted larger samples. Harvard single-beam trip balances were used for weighing samples because they were found convenient and accurate despite the constant motion of the boat. Wooden Petri dish boxes were used for storing and incubating plates. A plywood cabinet was used as an incubator during summer trips and an electric incubator was used during the winter months.

RESULTS AND DISCUSSION

Sampling: It was necessary to determine the smallest weight or least number of shrimp which would constitute a representative sample for accurate bacterial counts. Data (Table 1) indicate that 70 to 80 grams (about three headless shrimp) was the maximum capacity of the 500-ml. Waring Blendor bowl when the dilution water was added. Difficulty in pipetting thick blended samples and the small capacity of the container necessitated a dilution of one to four, or greater, in grinding samples. Plate counts on a composite two-pound market [3] sample of headless shrimp

[2] The words "freshly caught" refer to shrimp which have not been iced and which were plated within two hours after the net was emptied.

[3] The word "market" as used in this paper refers to a product purchased from wholesale or retail dealers. The consumer product is usually headless and may have been iced three to 15 days on trawlers and in transit to the dealer. Markets differ in their method of holding shrimp during the two or three days of sale. Some dealers pack the shrimp in ice and refrigerate them; others store shrimp in enamel containers in refrigerators.

reported (Table 1) indicated that the average of four samples of three shrimp gave results within the expected accuracy of the plate count. In ordinary routine work, the standard deviation expected in plate counts is ± 25 per cent, Prescott, Winslow, and McCrady (1946). The average of four samples of three shrimp each was 19,500,000 ± 28 per cent and the average of five samples of single shrimp, 25,500,000 ± 112 per cent. Limited time and facilities for work required that duplicate rather than quadruplicate samples be used for determining the count. Because of their size, single whole shrimp were used in some determinations. Aver-

TABLE 1

Effect of Weight and Number of Shrimp on Bacterial Plate Count

Sample No.[1]	Number of shrimp	Weight	Dilution water	Blendor dilution factor[2]	Bacterial count	Average count and standard deviation pct.
		gm.	*ml.*		*per gm.*	
A	5	121.3	200	2.6	Impossible to pipette	
B	4	83.0	200	3.4	Impossible to pipette	
C	3	55.0	250	5.5	20,700,000	
D	3	76.5	200	3.6	21,900,000	
E	3	39.5	200	6.1	11,600,000	
F	3	42.8	200	5.7	23,900,000	
C-F av.	3	53.4	.....	5.2	.....	19,500,000 ± 28%
G	2	32.0	200	7.2	24,000,000	
H	1	22.5	250	12.1	70,200,000	
I	1	21.0	200	10.5	2,700,000	
J	1	23.3	200	9.6	10,800,000	
K	1	20.0	200	11.0	36,200,000	
L	1	10.0	200	21.0	7,700,000	
H-L av.	1	19.4	.....	12.8	.....	25,500,000 ± 112%
Average, 10 samples, 19 shrimp: 2		34.3				23,000,000

[1] Market shrimp. [2] Calculated as follows: $\frac{\text{Weight of sample in grams + ml. dilution water}}{\text{weight of sample in grams}}$

age weight of the sample used in over 400 counts was 55.5 grams, which when diluted with 250 ml. of water in the mixing container gave a dilution of 5.5.

Mixing Methods: Shrimp flesh was mixed and diluted by two methods: (1) By cutting with sterile scissors, placing in a liter Erlenmeyer flask containing 50 grams of rough sand and a measured quantity of distilled water, and vigorously shaking for at least one minute; and (2) by grinding in a Waring Blendor with a measured quantity of dilution water. The question arose as to the comparableness of plate counts by these methods. (Waring Blendor bowls which could withstand steam sterilization could not be purchased in the early part of this work.) Two representative boxes of headless shrimp, selected as unmarketable at processing plant, were frozen. Three days after freezing, duplicate three-shrimp samples from each box were plated. Each sample was mixed first by hand-cut-

ting and shaking. A 10-ml. aliquot for counts on hand-cut samples was removed and the remaining mixture immediately disintegrated with a Waring Blendor. The difference obtained by the two methods was no greater than the variation between duplicate samples mixed by either method. Of the four samples, three counts were higher when mixed with the Waring Blendor. Agreement between duplicates when using the Waring Blendor was slightly better than in hand-cut samples. Some variation was undoubtedly due to the tremendous numbers of bacteria in these spoiled or decomposing shrimp. The average plate count on all hand-cut samples was 67,600,000, which is 85 per cent of the count obtained when these same samples were mixed in the mechanical blendor. This agreement between counts on duplicate samples mixed by different methods was closer than often obtained on duplicate samples mixed by identical methods (replicates in Table 1). The above results indicated that such counts could be compared, and consequently the Waring Blendor replaced hand-mixing in all subsequent work.

Dilution and Plating Procedures: The original dilution of shrimp flesh was made in a flask or blendor, as described above. If the sample was shaken by hand, mixing was always sufficiently vigorous to dislodge the intestinal contents from the "vein" along the back, and to wash out the abdominal and thoracic contents from the head, if whole shrimp were being examined. If mixed by a Waring Blendor, the samples in added dilution water were completely macerated in one minute. It was necessary to wait five to 15 minutes after blending before making further dilutions, in order to allow the fluid to separate from the foam. Small pieces of flesh settled in this time and the middle layer of fluid could be pipetted without introducing an error from foam or air bubbles. All samples (Table 1) were measured by pipette and weighed to determine the accuracy of using a 10-ml. sample in a 90-ml. water blank for a one to 10 dilution. Provided the intermediate layer of fluid had formed, pipetting this fluid was as accurate as weighing the samples. Further dilutions from the one to 10 bottle were made, using one-ml. samples in nine-ml. water blanks. The standard coliform test in lactose broth was run on certain samples, using one-ml. samples from the original blended sample and all succeeding dilutions. Three or more dilutions were plated in duplicate, using Bacto nutrient agar 1.5 per cent.

Various questions concerning the medium used for plating and the time and temperature of incubation were answered by work done in the laboratory at Baton Rouge, Louisiana. The prime purpose of such work was to establish a routine and simple method which could be used with the limited facilities available in the galleys of fishing vessels. The percentage of colonies which developed daily during five days of incubation at 22 to 28°C.(72 to 82°F.) was determined. The average of 24 frozen shrimp samples indicated that a three-day count represented 92 per cent of the total colonies developing in five days. Periods of incubation longer than three days were not only inconvenient and undesirable in work aboard the trawler but were unnecessary, since they did not materially increase the accuracy of the method.

The effect of the temperature of incubation on the bacterial count, demonstrated that 22 to 28°C. incubation gave a higher count than 37°C. (99°F.). The total number of colonies developing from 14 samples incubated at 37°C. was only 20 per cent of the total number developing when incubated at approximately 25°C.(77°F.). When a few samples were incubated at 21°C., approximately 25°C., and 37°C., the highest count was obtained at 25°C.

Bacto-tryptone glucose extract agar, recommended in determining the plate count of milk (but without added skimmed milk), was compared with Bacto-nutrient agar, 1.5 per cent, as plating medium. The number of colonies developing in each medium after three days' incubation at approximately 25°C. indicated that tryptone glucose extract agar was not significantly superior to nutrient agar. Incubation at 37°C. gave similar results, but 37°C. counts on both media were less than 25°C. Since the difference in counts obtained on the two media, incubated at either 25°C. or 37°C., was no greater than the difference between duplicate plates of the same sample, or between duplicate samples plated upon a single medium, all counts were calculated from nutrient agar plates.

Counting and Calculations: A Quebec Colony Counter was used for counter. Three-day plates containing 30 to 300 colonies were used whenever possible. The bacterial plate count was expressed as the number of bacteria per gram of shrimp. The blending or mixing dilution factor was calculated to one decimal place by the method indicated (footnote Table 1). In over 100 hand-cut samples, this factor averaged 5.7, and in approximately 300 blended samples was 5.4. In order to avoid fictitious accuracy, counts were corrected to two significant figures, as suggested in *Standard Methods for the Examination of Water and Sewage* (1938).

These results on the effect of time and temperature of incubation upon bacterial count are in agreement with Nickerson (1946), who stated that an incubation temperature of 25°C. had been found to give higher bacterial counts on frozen products than either 20°C. or 37°C. He also stated it was necessary to incubate Petri dish cultures for a period of 72 hours when nutrient agar was used as a culture medium.

SUMMARY

Methods of catching, handling, and storing shrimp on fishing vessels were recorded.

A method which could be used on board boats was developed for bacterial plate counts on shrimp. The minimum quantity for accurate bacterial plate counts was determined as duplicate 50-gram samples of three or more shrimp. It was found that mixing by hand-cutting or by a Waring Blendor gave comparable results. Incubation for three days at 25°C. gave more accurate counts than shorter periods or 37°C. incubation. Bacto-tryptone glucose extract agar was not significantly superior to Bacto-nutrient agar, 1.5 per cent, as a plating medium.

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BACTERIOLOGY OF SHRIMP.[1] II. QUANTITATIVE STUDIES ON FRESHLY CAUGHT AND ICED SHRIMP

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Shrimp-fishing methods and the procedures developed for bacteriological work on shrimp have been reported in the introductory paper of this series, Green (1949). The present report is concerned with the bacterial load of freshly caught shrimp and with some factors which affect the storage life of fresh shrimp before they reach the consumer market.

REVIEW OF LITERATURE

A search of the literature did not reveal any work on the numbers or kinds of bacteria found in fresh shrimp. Clark and MacNaughton (1917) stated that freshly caught shrimp should be headed, washed, and iced as quickly as possible because the dark liquid in the stomach consisted of oily, partially digested plant and animal material which readily decomposed. They emphasized that this liquid, as well as the body slime, must be removed by thorough washing before icing. Cameron and Williams (1934a,b) investigated procedures in shrimp canning which would eliminate spoilage. They did not report bacterial counts or the species isolated, but found that washed raw shrimp in canneries were heavily contaminated with bacteria. The practice of washing in tanks was inefficient because the water was not changed often enough, and sometimes the bacterial load on fresh shrimp was actually increased by washing. They recommended flume washing and other sanitary precautions which would prevent spoilage in canned shrimp.

Numerous papers on the bacteriology of fresh marine-fishery products were reviewed by Griffiths (1937). Conclusions which may be drawn from the literature are:

Excised muscle flesh of freshly caught fish is sterile, Fellers (1926), Harrison, Perry, and Smith (1926), Hunter (1920), Kiser and Beckwith (1944), Procter and Nickerson (1935), and Shewan (1944, 1945).

Bacteria on the surface of the skin and in the slime constitute the greatest percentage of the bacteria in fresh, iced, and spoiled fish, Bedford (1933a, 1933b), Fellers (1926), Schwartz and Zeiser (1939), and Shewan (1942).

Bacteria in the intestines and viscera of feeding fish may hasten spoilage, particularly if careless gutting injures the flesh or if fish are inadequately washed after gutting and before icing, Bedford (1933b), Dyer, Dyer, and Snow (1946), Kiser and Beckwith (1944), and Shewan (1942).

Other factors influencing the bacterial contents of fresh fish are the bacteria present in bottom mud, bilge water, sea water, and ice, Bedford (1933b), Sanborn (1930), and Shewan (1945).

[1] This project was supported in part by a grant from The Refrigeration Research Foundation, Berkeley, California.

The bacterial count increases steadily throughout iced storage and bacterial decomposition of fish flesh does occur at 0°C.(32°F.), Griffiths and Stansby (1934), Kiser (1944), Reay (1935), and Shewan (1944).

Widely varying bacterial counts are found on market fish; counts higher than 1,000,000 per gram of flesh have been considered as indicative of a product of poor quality, Fitzgerald and Conway (1937), Griffiths and Stansby (1934), Harrison *et al.* (1946), Hunter (1920), Hunter and Linden (1923), Reay (1935), and Shewan (1944).

EXPERIMENTAL PROCEDURE

All work on the preservation of shrimp before marketing was done aboard fishing vessels, on three different trips, by following procedures outlined in the previous paper. Unless otherwise noted, all samples were caught in the Gulf of Mexico, in outside waters. The experimental work followed the usual practices and methods of the shrimping industry; no unusual or different precautions were observed by the fishermen because of the bacteriological work in progress during the trips.

RESULTS AND DISCUSSION

Freshly Caught, Unwashed Whole Shrimp: Whole shrimp examined immediately after removal from the trawl-net varied in bacterial count from 1,600 to 1,200,000 per gram. Counts obtained on 22 samples are listed (Table 1). Since the first of six samples were caught in an inland bay, or in the Gulf of Mexico near the drainage from this bay, a more representative average count was obtained when these were omitted. This average of 42,000 per gram of whole shrimp was compiled from 14 one-shrimp samples caught in commercial fishing nets in widely different areas off the shore of Louisiana. The grade as used in the shrimping industry indicates the number of headless shrimp per pound. The average count of six samples of Grade 15 shrimp (largest size in Table 1) was 31,000. Five samples of Grade 25 averaged 75,000 per gram, and six samples of Grade 60 Gulf shrimp averaged 210,000. An inverse correlation existed between the size of the shrimp and the bacterial content. The ocean bottom along the coast of Louisiana within the areas listed (Table 1) is principally a smooth, packed mud. Off Barataria Bay and off Sabine Pass, a soft mud bottom is found in certain areas. A single plate count of the bottom mud at Trinity Shoals Buoy (eight fathoms of water) was 4,000 per gram. Surface sea water from the same location contained 24 bacteria per ml. No relationship was evident between the character of the bottom mud and the bacterial count on whole, unwashed shrimp. The difference between the average count of 350,000 on shrimp caught in the test-net, and the count of 76,000 when caught in the same area in the regular net, may be due to the longer time (two to three hours) involved in regular trawling, which provided more adequate washing than the short time (seven to 15 minutes) in which a test or try was made.

Effect of Washing and Heading Shrimp on Bacterial Count: Washing the shrimp reduced the number of bacteria present. (Unless otherwise noted, all shrimp on these trips were washed with a hose.) Washing headless or whole shrimp from the same catch produced an average reduction of 42 per cent and a range from zero to 98 per cent reduction. An average

TABLE 1

Bacterial Plate Counts on Whole, Unwashed, Freshly Caught Shrimp

Sample No.	Number of shrimp	Weight	Grade	Bacteria	Average of replicate samples	Location of fishing	Off shore	Water depth
		gm.	*ct./lb.*	*per gm.*			*mi.*	*fathoms*
19	?	50	60+	1,200,000		Inside Barataria Bay	....	3/4
19d	?	50	60+	1,200,000	1,200,000	Inside Barataria Bay	....	3/4
31	4	50	60	280,000		Gulf off Barataria Bay	20	20
32	4	50	60	410,000[1]		Gulf off Barataria Bay	20	20
33	4	50	60	370,000	350,000[1]	Gulf off Barataria Bay	20	20
34	4	50	60	150,000		Gulf off Barataria Bay	20	20
35	4	50	60	43,000		Gulf off Barataria Bay	20	20
36	4	50	60	34,000	76,000	Gulf off Barataria Bay	20	20
1	1	31	25	140,000		Trinity Shoals	22	6
2	1	30	25	190,000	160,000	Trinity Shoals	22	6
7	1	45.5	15	26,500		SSE Cameron	25	8
8	1	43	15	3,100	15,000	SSE Cameron	25	8
21	1	47.5	15	27,500		Trinity Shoals	22	9
27	1	29	25	27,000		Trinity Shoals	22	9
28	1	28.5	25	11,000	19,000	Trinity Shoals	22	9
6-1	1	29	25	4,800		Off Marsh Island	12	2
6-2	1	24	30	15,000	9,900	Off Marsh Island	12	2
6-7	1	51.5	15	98,000		Trinity Shoals	28	10
6-8	1	36	20	22,000	60,000	Trinity Shoals	28	10
6-12	1	44.5	15	4,600	.....	Trinity Shoals	28	10
6-14	1	34.5	20	1,600	.....	Trinity Shoals	28	10
6-39	1	58.8	15	24,000	.....	S.E. La. Point Sabine Pass	8	6
Av. 22 samples		42.4	25	190,000				
Av. Gulf shrimp		41.6	20	94,000 (excluding 19 and 19d from inside waters)				
Average		38	20	42,000 (excluding all inland and Barataria Bay area samples)				

[1] Caught in test-net.

of three samples of whole, freshly caught, washed shrimp was 16,000 bacteria per gram; a 62 per cent reduction from the 42,000 average (Table 1) on unwashed shrimp caught at different times during two fishing trips.

Bacterial counts on headless, unwashed shrimp were lower than on whole shrimp from the same catch. Removal of the heads by cutting aseptically from whole shrimp lowered the average count from 76,000 to 17,000 on three samples. Whole shrimp of 27,500 count, when headed had only 8,800 bacteria per gram of "tail." Two samples of the removed heads averaged 31,000 bacteria per gram. The heads constituted about 40 per cent of the weight of the whole shrimp, but contained about 75 per cent of the bacteria present, if the 42,000 average count on whole shrimp (Table 1) is compared with 10,000 average count on headless shrimp.

A single count on 0.2 gram of slime removed from one whole and four headless unwashed shrimp was 210,000 bacteria per gram. The average of two counts on the headless shrimp from which the above slime had been partially removed was 15,000. Comparison of counts on the slime and on headless shrimp indicated bacterial count of freshly caught, headless shrimp was largely a determination of the bacteria on the surface and adhering debris, and further emphasized the importance of adequate washing before storage.

Bacterial counts on the product as prepared for icing under commercial conditions, headed by the fisherman before washing, were bacteriologically cleaner than any samples of freshly caught shrimp. The average count, omitting samples caught in the Bay area of drainage, was 7,400. The lowest count was 200 bacteria per gram of washed headless shrimp, and nine of the 14 samples were below 4,000 bacteria per gram.

The intestinal tract of the "tail" was examined for total numbers of bacteria, in an effort to determine the source of bacteria in washed shrimp other than those present in the slime. The intestines were removed aseptically from two headless shrimp, and a count of 7,000 bacteria per intestinal tract was found. The count on headless shrimp of the same catch was 51,000 per gram; only one-seventh of which may be estimated as present in the intestines. The canal constitutes only about 0.4 per cent of the weight of headless shrimp and obviously did not contribute a large per cent to the bacterial count of "tails."

Iced Headless Shrimp: Washed headless shrimp were packed between layers of ice as previously described, and bacterial counts from the bottom layers compiled (see Table 2). In the counts of Experiment 1, each sample consisted of three headless shrimp and averaged 67 grams. Counts rose steadily throughout the holding period despite adequate icing. The highest single count, 1,200,000 per gram, recorded on the sixth day, was not accompanied by any change in the odor or appearance of the shrimp. When unloaded at the end of the ninth day, the shrimp were an acceptable market product and superior in appearance to those usually purchased as fresh shrimp in inland Louisiana cities. A few shrimp, selected as spoiled when they were inspected in the purchasing house, contained 73,500,000 bacteria per gram after three days' frozen storage.

The successful use of certain germicidal ices, in improving the quality of fish, suggested the incorporation of bacteriostatic chemicals in the

crushed ice used for storing shrimp. The literature on this subject has been recently reviewed by Dunn (1947). Ice containing 0.05 per cent of a commercial preparation [2] was used in Experiment 2 (Table 2). Duplicate two-shrimp samples, averaging 58.3 grams, were removed daily from the bottom layer of headless shrimp. Results did not indicate any improvement in keeping quality, as judged by bacterial number. Tarr (1946) found that ice containing a commercial preparation of the same chemicals brought about only a very slight improvement in keeping quality of fish. He pointed out that some germicidal ices were more effective if the flesh of fish was slightly acid. The pH of the flesh of shrimp has been reported

TABLE 2

Bacterial Plate Counts on Iced, Headless, Washed Shrimp, Bottom Layer of Storage Bins

Description of sample	Experiment 1 Stored in ordinary ice		Experiment 2 Stored in ice containing a bacteriostatic agent[1]	
	Bacteria	Average of duplicate samples	Bacteria	Average of duplicate samples
	per gm.		*per gm.*	
Headless, washed, freshly caught....	7,300		650	
	10,500	8,900	1,600	1,100
Iced storage:				
6 hours.....	58,000			
	160,000	110,000	.....	.....
1 day.....	51,000		53,000	
	67,000	59,000	16,000	34,500
2 days.....	91,000		17,000	
	140,000	120,000	31,500	24,000
3 days.....	130,000		120,000	
	190,000	160,000	150,000	140,000
4 days.....	340,000		340,000	
	650,000	500,000	490,000	420,000
5 days.....	120,000		1,200,000	
	77,000	99,000	880,000	1,000,000
6 days.....	1,200,000		1,200,000	
	200,000	700,000	2,100,000	1,600,000
7 days.....	600,000	.....	600,000	
			330,000	460,000
8 days.....	.....	.....	6,800,000	.....
10 days.....	.....	.....	10,000,000	.....

[1] Labeled: "Active ingredients, chloramine-T 0.00016 and sodium benzoate 0.00032 by volume" when 68.3 grams are incorporated in 300 pounds of ice.

[2] Labeled: "Active ingredients, chloramine-T 0.00016 and sodium benzoate 0.00032 by volume" when 68.3 grams are incorporated in 300 pounds of ice.

as 7.44 when freshly caught and 8.18 after six days' iced storage, Fieger, Green, Lewis, and Kaloyereas (1947).

Spoiled Shrimp: Counts on spoiled shrimp are compiled (Table 3) for comparison with marketable shrimp in previous tables. Headless Samples 1-16 through 1-27 contained 51,000 bacteria per gram when freshly caught, and Whole Samples 1-11 through 1-28 had a count of 48,500 before spoilage.

TABLE 3

Bacterial Plate Counts on Spoiled Shrimp

Sample No.	Description and history of sample	Bacteria
		per gm.
46	From same boatload as Experiment 1, Table 5 and Experiment 3, Table 7	131,600,000
47	Headless shrimp, iced 3 to 9 days, rejected as spoiled because of red color or crushed, torn condition on inspection in purchasing plant. Frozen and held in frozen storage for 3 days until counts could be made.	64,700,000
48		84,200,000
49		103,800,000
50		29,500,000
51		44,700,000
52		82,700,000
53		46,700,000
Average of 46-53, composite spoiled sample		73,500,000
1-16	Headless shrimp, held 24 hours at 20° to 24°C.	23,200,000
1-29	Headless shrimp, held 48 hours at 20° to 24°C.	980,000,000
1-15	Headless shrimp, held 24 hours at 30° to 35°C.	5,000,000
1-27	Headless shrimp, held 48 hours at 30° to 35°C.	858,000,000
1-11	Whole shrimp, held 24 hours at 20° to 24°C.	28,000,000
1-30	Whole shrimp, held 48 hours at 20° to 24°C.	396,000,000
1-10	Whole shrimp, held 24 hours at 30° to 35°C.	80,400,000
1-28	Whole shrimp, held 48 hours at 30° to 35°C.	876,000,000
Average, 12 samples of headless spoiled shrimp		288,000,000
Average, 4 samples of whole spoiled shrimp		345,000,000

Iced Whole Shrimp: Whole shrimp, packed in ice, were not held longer than four days before unloading. Bacterial counts during the storage period are presented (Table 4). In Experiment 3, samples of single whole shrimp, averaging 32.4 grams, were used for determining the count. In order to determine the relative numbers of bacteria in the headless (edible) part only, samples averaging 48.8 grams and consisting of three aseptically removed "tails" were used. The tail flesh did not show the same rate of bacterial multiplication which occurred in whole shrimp. At the end of two days' storage, counts on whole shrimp had increased seven-fold; on headless shrimp five-fold. The removed heads from whole shrimp iced one day contained 800,000 bacteria per gram; after two days, 600,000 per gram; figures two to four times higher than tail flesh at the same times. This difference was not unexpected in view of previous findings, and since the largest part of the digestive tract was present in the removed heads. Harrison *et al.* (1926) noted that carefully gutted and washed fish kept better than whole fish. Dyer *et al.* (1946) found that bacterial counts and chemical tests on gutted cod stored in melting ice showed that spoilage

TABLE 4

Bacterial Plate Counts on Iced, Whole, Washed Shrimp, Bottom Layer of Storage Bins

Description of sample	Experiment 3 Stored in ordinary ice	Experiment 4 Stored in ice containing a bacteriostatic agent[1]	
	Bacteria	Bacteria	Average of duplicate samples
	per gm.	*per gm.*	
		500	.....
Whole, washed, freshly caught	46,500	600	550
Iced 1 day	480,000[2]	17,000	
		16,000	16,500
Iced 2 days	330,000	28,000	
		21,000	24,500
Iced 4 days	.....	140,000	.....
Same samples as above, headed aseptically:			
Headless, washed, freshly caught	54,000	.....	.....
Headless, iced 1 day as whole shrimp	240,000	.....	.....
		35,000	
Headless, iced 2 days as whole shrimp	260,000	52,000	43,500
Headless, iced 4 days as whole shrimp	.....	94,000	.....

[1] Labeled: "Active ingredients, chloramine-T 0.00016 and sodium benzoate 0.('32 by volume" when 68.3 grams are incorporated in 300 pounds of ice. [2] Average of two counts.

originated in the belly cavity and that bacteria did not penetrate muscle until spoilage was well advanced.

Ice containing chloramine-T and sodium benzoate did not prevent the steady increase in bacteria during the short icing period (Experiment 4, Table 4). After four days' iced storage, the bacterial count of whole shrimp had increased 255-fold.

Shrimp From Different Layers of the Icing Bins: Since the oldest shrimp were present in the bottom layer, and melting ice from added upper layers washed down over lower layers, a study of the influence of position in the bin on bacterial count was indicated. In nine days, headless shrimp in the layer next to the top increased from 1,800 bacteria per gram to 2,400 per gram; whereas the bacteria in bottom-layer shrimp increased 1,000-fold. Whole shrimp showed a similar difference; the bottom layer contained 250 times, and the upper layer only 125 times, as many bacteria as the freshly caught whole samples of 550 count. Water draining through a different bin of one-day-iced whole shrimp contained 5,900,000 bacteria per ml.

The unused crushed ice stored in the hold may have contributed to the bacterial content of iced shrimp. Ice stored for two days on one boat contained 85,000 bacteria per gram; on another boat after seven days' storage, the unused ice contained 230,000 bacteria per gram.

Refrigeration of Shrimp Without Direct Contact With Ice: In preliminary work samples of headless and whole shrimp stored in a closed hydrator in an electric refrigerator 2°C.(36°F.) did not show the same increases in bacterial numbers as those packed in layers of ice. Further work was undertaken to determine effect of refrigerated storage on bacterial count. Washed, headless shrimp were stored in heavily-waxed, two-piece, telescope cardboard cartons of sufficient size to hold five pounds of shrimp. These boxes were placed between layers of crushed ice near the top of an ordinary icing bin. Results of two such experiments are presented (Table 5). At the end of each storage period, the shrimp did

TABLE 5

Bacterial Plate Counts on Headless Shrimp Refrigerated in Boxes Stored in Crushed Ice

Description of sample	Experiment 5	Experiment 6	
	Bacteria	Bacteria	Average of duplicate samples
	per gm.	*per gm.*	
Headless, washed, freshly caught	7,000	3,800	.....
Stored 1 day	230,000	1,900	
		3,000	2,500
Stored 2 days	10,500	3,300	
		5,600	4,400
Stored 3 days	.....	4,000	
		9,200	6,600
Stored 4 days	.....	4,600	
		15,000	9,800
Stored 5 days	.....	2,200	
		2,200	2,200
Stored 6 days	.....	900	
		4,300	2,600
Stored 7 days	.....	2,700	
		3,700	3,200

not show visible evidence of dehydration and were an excellent product as judged by odor and appearance. The only objectionable feature was the presence of black or dark spots at the edges of shell segments. This darkening did not occur to the same degree in shrimp iced in the usual manner. The remarkable constancy of the count throughout seven days of such storage may be dependent on a number of factors. In iced storage, the bacterial load of shrimp in the bottom of a bin was increased by the percolation of melting ice from upper layers, and some injury to flesh occurred because of the pressure of upper layers. These influences were absent in the boxed shrimp experiments. According to Lemon (1946), during the past few years refrigerated storage rooms at temperatures between 0 and 2°C.(32 and 35°F.) have been devised for holding fish.

Only sufficient ice was used in the containers of fish to keep them moist, he reported. He suggested that such refrigeration should be more generally used, since it increased the storage life of fresh fish. Tarr (1945) found that bacteria in dehydrated fish increased only at 85 per cent relative humidity or higher, and that viable bacteria decreased slowly in samples stored at relative humidities too low to support their active multiplication.

Market Shrimp: In an effort to evaluate refrigeration facilities used in transit from the fishing vessels to the consumer markets, a number of market samples were examined bacteriologically. Results are presented (Table 6). Average count of 32,000,000 was higher than any obtained on shrimp while aboard the trawlers. The lowest count of 600,000 was recorded on shrimp which had not yet passed through the purchasing house:

TABLE 6

Bacterial Plate Counts on Market Samples of Fresh Headless Gulf Shrimp

Sample No.	Description of sample	Bacteria	Average of replicate samples
		per gm.	
C-L	Purchased from Baton Rouge retail dealer; supposedly caught in Gulf of Mexico off Mississippi Coast. Individual counts reported (Table 1, previous paper).	range from 2,700,000 to 70,200,000	.....
	Average of ten samples		23,000,000
201	Purchased from New Orleans wholesale dealer as soon as unloaded from fishing boat.	700,000	
202		500,000	600,000
231[1]	Purchased from Baton Rouge retail dealer on arrival from Houma, La.	161,700,000	
232[1]		98,000,000	129,900,000
233	Purchased from Baton Rouge retail dealer on arrival, probably from Golden Meadow, La.	13,500,000	
234		11,400,000	12,500,000
235	Purchased from Baton Rouge retail dealer on arrival from Morgan City, La.	3,600,000	
236		3,700,000	3,700,000
251[1]	Purchased from Baton Rouge retail dealer on arrival from New Orleans, La.	161,200,000	
252[1]		58,000,000	119,600,000
255[2]	Purchased from wholesale dealer, French Market, New Orleans, La.	850,000	
256[2]		1,400,000	1,100,000
267	Purchased from Baton Rouge retail dealer on arrival; caught near S.W. Pass of Mississippi River	6,900,000	
268		9,200,000	8,000,000
273[2]	Purchased from wholesale dealer, French Market, New Orleans, La.	3,600,000	
274[2]		2,000,000	2,800,000
Average of 26 samples			32,600,000
Average of 22 samples of good quality			13,000,000

[1]A sample of questionable acceptance by organoleptic examination. [2]Arrived as whole shrimp, appearance and condition excellent and similar to the appearance of freshly caught shrimp; headed by hand before plating.

other low counts of 1,100,000 and 2,800,000 were also obtained on shrimp which had not reached retail channels. Three samples of shelled, washed meat purchased from a wholesale dealer in New Orleans, Louisiana, averaged 280,000 bacteria per gram. This average further emphasized that bacteria in slime and debris on the shell surface probably constituted more than one-half the total count on the market product. With the exception of Samples 231-232, which had a strong fishy odor, and 251-252, which had a marked odor of ammonia, all samples were acceptable organoleptically. The average count of 13,000,000 on good quality market shrimp was higher than the standard of 1,000,000 per gram of marketable fish suggested by Griffiths and Stansby (1934).

SUMMARY AND CONCLUSIONS

Bacterial counts on fresh shrimp in all stages from the time caught, through heading, washing, and icing until delivery in consumer markets, have been recorded.

Fourteen representative samples of freshly caught, whole Gulf shrimp averaged 42,000 bacteria per gram. The majority of these bacteria were present in the surface slime and in the "heads" of shrimp. Heading and thorough washing reduced the bacterial count per gram by approximately 75 and 40 per cent, respectively. Thirteen samples of freshly caught, headless, washed shrimp contained an average of 7,400 bacteria per gram.

The bacterial count during seven to 10 days' iced storage in the bottom layer of the bin increased steadily to a million, or more per gram. Shrimp packed in upper layers of an icing bin had much lower counts than those of similar age in the bottom layers. Use of ice containing chloramine-T and sodium benzoate did not decrease the actual number, or diminish the rate of multiplication, of bacteria. Average count on two composited samples from one trawler, when unloaded, was 55,000. Spoiled shrimp varied widely in total numbers of bacteria, 12 samples of headless spoiled shrimp averaging 288,000,000.

Refrigeration without direct contact with ice produced a superior product, bacteriologically; the count after seven days' storage approximated the freshly caught product and indicated that use of mechanical refrigeration should be investigated further.

Twenty-six samples of market shrimp, purchased from wholesale and retail dealers, averaged 32,600,000 bacteria per gram. A comparison of this figure with those on freshly caught and iced shrimp indicated that better refrigeration methods are needed in transit to market, as well as on fishing boats.

The necessity of heading and thoroughly washing shrimp before icing, and washing at unloading and when re-icing, cannot be too strongly recommended for increasing the storage life of fresh shrimp.

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BACTERIOLOGY OF SHRIMP.[1]
III. QUANTITATIVE STUDIES ON FROZEN SHRIMP

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Certain factors affecting the quality of frozen shrimp were pointed out by Fieger and DuBois (1946). Fieger, Green, Lewis, and Kaloyereas (1947) emphasized that pre-freezing age and condition were reflected in the frozen product, and discussed tests for the quality of shrimp. Quantitative bacteriological data on freshly caught and iced shrimp were presented in the second paper of this series, Green (1949b). Bacterial counts on frozen shrimp during different periods of storage at —17.8°C.(0°F.) are recorded in the present paper.

REVIEW OF LITERATURE

No work on bacteriology of frozen shrimp was found in the literature, but a number of investigators have reported similar work on frozen fruits and vegetables, and a few workers have studied the microbiology of frozen fish.

It has been established that some bacteria are destroyed by freezing and frozen storage. Literature on the speed with which these bacteria are destroyed is controversial. Haines (1937-38) found that various freezing temperatures produced essentially the same reduction in suspensions of bacteria, but that prolonged storage at various temperatures resulted in the greatest death rate at the higher temperatures, —2°C.(28°F.) being the most destructive. Weiser and Osterud (1945), in extensive investigations, concluded that death by freezing involved a rapidly acting or "immediate" death, caused by freezing and thawing *per se*, and a "storage" death, which is a direct function of time and temperature. They also concluded that death by freezing was marked, but did not vary with the intensity of the freezing temperature. They noted t' at repeated fluctuations of temperature below freezing did not exert a lethal action in addition to that of storage.

Prescott, Bates and Highlands (1932) reported that bacteria in commercially quick-frozen haddock, did not decrease in number during 25 weeks at —4, —6.6, —12, and —18°C.(25, 21, 12, and —1°F.). They found bacterial count in the fat of a similarly treated frozen sample of lamb chops was reduced slightly, the greatest decrease occurring at —6.6°C. (21°F.). Gibbons (1934) found bacterial reduction due to freezing "ice fillets" was little changed by storage at —18°C.(—1°F.) for one year. Stewart (1934) recommended —6°C. or below as the storage temperature necessary to prevent bacterial decomposition of fish during the period of storage in use commercially. She reported that pieces of fish muscle inoculated with bacteria present in haddock slime showed growth at —2, —6,

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and —12°C., the latter two finally decreasing after the initial increase, Stewart (1933). Kiser and Beckwith (1942) found sharp reductions in the flora of muscle and intestinal contents of mackerel due to freezing and storage at —28°C.(—18°F.) for 10 days. Freezing and storage for 48 hours at —28°C. produced greater decreases in suspensions of bacteria isolated from mackerel than did similar treatment at —20°C.(—4°F.); the *Achromobacter sp.* was reduced 91.9 per cent and the *Micrococci*, 53.4 per cent. Kiser (1943) showed that during the first 300 hours of freezing at —28°C., the destruction of a suspension of *Achromobacter* was proportional to the number of viable organisms present at any given time. During subsequent periods, up to 1,200 hours, no such relationship existed, and a total sterilization did not always occur.

EXPERIMENTAL PROCEDURE

Headless shrimp of known history, Green (1949b), were repacked in ice as soon as the bins of the fishing vessels were opened for unloading, and transported to this laboratory for freezing on the same day. Before freezing, all samples were washed in ice water and packed in laminated and waxed one-piece telescope cartons of sufficient size to hold 300 to 400 grams of shrimp. These were overwrapped with laminated glassine and frozen at —17.8°C.(0°F.); freezing was completed in from six to eight hours. A few samples were frozen in a plate freezer at —34.4°C.(—30°F.) in approximately two hours and removed after one to two days to —17.8°C. (0°F.) storage. Samples were held in storage for varying periods up to one year.

Commercially frozen shrimp were obtained immediately after freezing and after different periods of frozen storage. Most wholesale dealers blast-freeze shrimp in five-pound cartons for restaurant trade. These are glazed within two days after freezing and are reglazed, if necessary, during storage. The temperature of commercial frozen storage is —17.8°C.(0°F.), or occasionally lower. Samples of fresh market shrimp were purchased from wholesale and retail dealers, frozen after various treatments, and stored in this laboratory for periods up to two months.

A single box of frozen shrimp was removed at designated intervals of time, and was allowed to thaw only enough to separate duplicate or more samples of at least three headless shrimp each. Each sample weighed at least 50, and not more than 100, grams. Methods of sampling, mixing, and making bacterial plate counts were discussed in the first paper of this series, Green (1949a).

RESULTS AND DISCUSSION

Shrimp Frozen Immediately After Heading and Washing: Bacterial plate counts on headless shrimp frozen in a plate freezer at —34°C. (—30°F.) within three hours after caught are presented (Table 1). Two days after freezing, the samples were placed in —17.8°C.(0°F.) storage. These shrimp were caught in an area near extensive land drainage and were washed in hand-nets by dipping into barrels of water from a bay. This may partially explain the high count of 160,000 per gram when freshly caught, and the variations among individual one-pound samples.

TABLE 1

Bacterial Plate Counts on Shrimp Frozen Immediately[1] After Heading and Washing

Description of sample[2]	Bacteria	Reduction
	per gm.	*pct.*
Before freezing	160,000	....
Frozen storage:		
1 month	60,000	62
2 months	46,000	71
3 months	51,000	68
4 months	20,500	87
5 months	83,000[3]	48
6 months	370,000	....
9 months	42,000	74
12 months	22,000	86

[1]Three hours were required for heading and washing. [2]Each sample weighed 454 grams, contained 54 to 62 shrimp, and was hand-cut into 908 ml. water. [3]Average of two samples of 83,000 and 82,000 count.

Headless Shrimp Frozen After Storage in Icing Bins: Bacterial plate counts on frozen shrimp from two different icing bins are recorded (Table 2). Samples in Experiments 1 and 2 are from the same bottom layers recorded in Table 2 of the second paper of this series, Green (1949b) and iced nine to 10 days before freezing. Since the count of 600,000 (Experiment 1) was recorded on the seventh day (before freezing on the ninth day), it was impossible to calculate percentage reductions due to freezing or to frozen storage. These figures of more than a million bacteria per gram throughout 12 months' frozen storage were the highest obtained on any samples from three fishing trips.

In Experiment 2 (Table 2) percentage reductions obtained by frozen storage were remarkably constant between two and 12 months and ranged from 89 to 99.2 per cent. Since bacterial increases on these bottom-layer shrimp before freezing were not prevented by storage in ice containing a bacteriostatic agent,[2] it is questionable whether such treatment influenced the counts during frozen storage. No adequate explanation of these lower counts can be given at this time.

Bacterial counts on headless shrimp which had been iced for two days in the top layer of a bin are recorded (Table 2). No significant differences were evident between counts on the second and fifth months. Total number of bacteria present at the end of five months of frozen storage indicated that these samples before freezing were a cleaner product bacteriologically than those in Experiments 1 and 2.

Shrimp Headed and Frozen After Icing as Whole Shrimp: Bacterial counts on headless shrimp, frozen after they were stored four days as whole shrimp in the bottom layers of icing bins, are recorded (Table 3). Bacterial counts before icing were given (Table 4, second paper of this series). Shrimp frozen for Experiments 3 and 4 were obtained from two different fishing trips; in the latter, ice containing the bacteriostatic agents previously mentioned was used. Counts recorded in Experiment 4 compare

[2]Labeled: "Active ingredients, chloramine-T 0.00016 and sodium benzoate 0.00032 by volume" when 68.3 grams are incorporated in 300 pounds of ice.

TABLE 2

Bacterial Plate Counts on Headless Shrimp Frozen and Stored at at —17.8°C.(0°F.) After Iced Storage

Description of sample	Bottom layers of icing bins					Top layers of icing bins	
	Experiment 1		Experiment 2				
	Bacteria	Average of replicate samples	Bacteria	Average of replicate samples	Reduction	Bacteria	Average of replicate samples
	per gm.		*per gm.*		*pct.*	*per gm.*	
Freshly caught	.....	8,900	.....	1,100	.....	.....	1,400[3]
Before freezing	.....	600,000[1]	.....	10,000,000[2]	.....	.....	.....
Frozen storage:							
2 months	7,000,000		480,000			72,000	
	15,800,000	11,400,000	750,000	600,000	94.0	43,000	58,000
3 months	16,500,000		130,000			21,000	
	4,400,000	10,400,000	110,000	120,000	98.8	37,500	29,500
4 months	4,200,000		650,000			40,500	
	3,200,000	3,700,000	450,000	550,000	94.5	39,000	40,000
5 months	5,900,000		340,000			28,000	
	4,500,000	5,200,000	500,000	420,000	95.8	58,000	43,000
6 months	11,700,000		950,000				
	10,000,000	10,800,000	1,300,000	1,100,000	89.0	.....	.....
7 months	18,300,000		960,000				
	7,500,000	12,900,000	230,000	600,000	94.0	.....	.....
8 months	3,400,000		370,000				
	5,200,000	4,300,000	65,000	220,000	97.8	.....	.....
9 months	1,800,000		120,000				
	2,200,000	2,000,000	280,000	200,000	98.0	.....	.....
			300,000				
10 months	2,500,000		93,000	180,000	98.2	.....	.....
	1,500,000	2,000,000	160,000				
11 months	5,600,000						
	8,800,000		70,000				
	1,800,000		100,000	85,000	99.2	.....	.....
	2,900,000	4,800,000					
12 months	1,100,000						
	2,100,000	1,600,000	.....	.....	.....	.....	.....

[1]Count on seventh day, frozen on ninth day of iced storage. [2]Count on 10th day, frozen on 10th day of iced storage. [3]Average of seven samples of freshly caught shrimp; no counts recorded during iced storage, frozen on second day of iced storage.

favorably with those on shrimp frozen immediately after heading and washing, but it is evident that the pre-freezing count in Experiment 4 was lower than in Table 1.

Commercially Frozen Shrimp: Bacterial counts on commercially frozen shrimp, held in iced storage prior to freezing, are presented (Table 4). Samples in Experiment A were obtained from the dealer who purchased

TABLE 3

Bacterial Plate Counts on Shrimp Headed and Frozen After Four Days' Iced Storage in Bottom Layers of Bins

Description of sample	Experiment 3		Experiment 4		
	Bacteria	Average of replicate samples	Bacteria	Average of replicate samples	Reduction
	per gm.		*per gm.*		*pct.*
Freshly caught, headless	.....	54,000[1]	.....	.....	.....
Before freezing	.....	260,000[1,2]	.....	94,000[1,3]	.....
Frozen storage:					
2 months	6,600,000		22,500		
	4,300,000	5,500,000	33,500	28,000	70.2
3 months	1,300,000		27,500		
	850,000	1,100,000	29,000	28,500	69.7
4 months	1,000,000		20,500		
	900,000	950,000	34,000	27,500	70.7
5 months	2,000,000		18,500		
	1,900,000	2,000,000	21,000	20,000	78.7
6 months	620,000		13,000		
	550,000	600,000	23,500	18,500	80.3
7 months	550,000		41,500		
	700,000	650,000	29,000	35,500	62.2
8 months	650,000		12,000		
	600,000	650,000	25,500	19,000	79.8
9 months	650,000		40,000		
	320,000	480,000	34,500	37,000	60.6
10 months	11,700,000		25,500		
	220,000	6,000,000	34,500		
			31,000		
			34,500	32,500	65.4
11 months	300,000				
	170,000				
	90,000		23,500		
	180,000	180,000	13,000	18,500	80.3
12 months	88,000		.....		
	54,000	71,000	.....	.....	.....

[1] Single sample used for calculating count. [2] Count on headless shrimp after icing two days as whole shrimp; headed and frozen on fourth day of iced storage. [3] Count on headless shrimp after icing four days as whole shrimp; headed and frozen on fourth day of iced storage.

the entire boatload on which previous work was recorded (frozen samples in Experiment 1, Table 1, and Experiment 3, Table 6 of this paper). The commercial product was sent to this laboratory for frozen storage immediately after freezing and glazing. Duplicate samples were removed from a five-pound box (with as little thawing as possible) at intervals of one month. On the eighth month, counts were also made from a previously

TABLE 4

Bacterial Plate Counts on Commercially Frozen Headless Shrimp

Description of sample[1]	Experiment A		Experiment B	
	Bacteria	Average of replicate samples	Bacteria	Average of replicate samples
	per gm.		*per gm.*	
Freshly caught, average of 6 samples	.....	13,500	.....	.....
Frozen storage:				
1 week	.....	.....	550,000	
			490,000	
			600,000	550,000
2 months	3,800,000		570,000	
	2,900,000	3,400,000	360,000	460,000
3 months	1,400,000	.....	.....	.....
4 months	1,700,000		190,000	
	4,600,000	3,200,000	320,000	260,000
5 months	4,600,000			
	11,200,000	8,300,000	.....	.....
6 months	5,400,000			
	37,300,000	22,000,000	.....	.....
7 months	6,700,000			
	20,000,000	12,400,000	.....	.....
8 months	4,700,000			
	3,600,000	3,000,000	.....	.....
8 months[2]	550,000			
	950,000	750,000	.....	.....
9 months	4,200,000			
	8,000,000	6,300,000	.....	.....
10 months	2,400,000			
	1,000,000	1,700,000	.....	.....
11 months	440,000			
	420,000	430,000	.....	.....
12 months	300,000			
	330,000			
	600,000	410,000	.....	.....

[1]Frozen after from four to 12 days' iced storage. [2]A previously unopened five-pound box used for this and all succeeding samples. Prior to this count, all samples were removed from the same five-pound box.

unopened five-pound box. The counts during storage were higher than those on laboratory frozen samples (Tables 1 and 6) and on some commercially frozen samples (Table 7). Variations in bacterial counts were possibly influenced by the fact that the commercial sample was composited from shrimp of different layers and ages (four to 12 days) and was not frozen as soon after unloading the trawler as were the laboratory samples.

Counts on another commercially frozen five-pound sample of shrimp from a different boat are recorded (Table 4, Experiment B). These shrimp were trucked to the freezing plant one day after unloading from the trawler, frozen on the third day, and placed in storage in this laboratory three days after freezing. The lower counts obtained indicated more expeditious handling or a cleaner initial product than used in Experiment A.

Frozen Headless Shrimp Refrigerated Before Freezing: The lowest bacterial counts during frozen storage were obtained on headless shrimp which before freezing had been refrigerated in waxed cardboard boxes

TABLE 5

Bacterial Plate Counts on Frozen, Headless Shrimp Refrigerated Before Freezing

Description of sample	Experiment 5		Experiment 6	
	Bacteria	Average of replicate samples	Bacteria	Average of replicate samples
	per gm.		*per gm.*	
Freshly caught, washed	.....	7,000[1]	.....	3,800[1]
Before freezing	.....	10,500[1,2]	2,700	
			3,700	3,200[2]
Frozen storage:				
2 months	350,000		13,500	
	70,000	210,000	6,000	9,800
3 months	110,000		4,100	
	100,000	100,000	4,500	4,300
4 months	80,000		1,100	
	34,500	57,000	1,700	1,400
5 months	13,500		850	
	11,000	12,500	800	850
6 months	26,000		3,300	
	36,500	31,500	6,300	4,800
7 months	.....		950	
	.....	.....	800	900
8 months	.....		325	
	.....	.....	600	450
11 months	14,000			
	16,500			
	85,000		.....	
	79,000	49,000	.....	.....
12 months	26,000			
	13,500			
	59,000		.....	
	160,000	65,000	.....	.....

[1] Single sample used for calculating count. refrigeration without direct contact with ice.
[2] Count after second day, frozen on fourth day of
[3] Count after seventh day, frozen on ninth day of refrigeration without direct contact with ice.

without direct contact with ice (Table 5). Experiments 5 and 6 in this table report data on the same shrimp as were used for results in Table 5, second paper of this series. During frozen storage, these shrimp developed more discoloration than any other product obtained directly from the fishing vessels. In over one-half the shrimp, brown or black spots appeared at the edges of the segments, and the normal bluish-white appearance of the shell changed to light brownish or greenish-yellow.

TABLE 6

Bacterial Plate Counts[1] on Market Shrimp

Pre-freezing treatment	Treatment	Bacteria before freezing	1 day's storage —17.8°C. (0°F.)	2 months' storage —17.8°C. (0°F.)
	pct. reduct.	*per gm.*	*pct. reduct.*	*pct. reduct.*
None	.....	129,900,000	66	93
None	.....	119,600,000	37	81
None	.....	12,500,000	57	82
None	.....	8,000,000	52	75
None	.....	3,700,000	11	78
None	.....	2,800,000	.....[2]	79
None	.....	1,100,000	23	44
Average	.....	.....	52	87
Washed one minute in pan of tap water or washed on trawler	52	3,800,000	49	32
	39	1,700,000	41	79
	.....	160,000[3]	.....	71[3]
	.....	94,000[7]	.....	70
	.....	51,000[7]	56	.....
Average	46	.....	44	41
Washed one minute in pan of water containing 0.05 pct. commercial preparation[4]	70	36,300,000	.....[5]	53
	.....	10,000,000[7]	.....	94
	70	2,400,000	0	77
	9	1,000,000	57	94
	89	320,000[7]	.....	.....
Average	70	.....	.....[6]	62
Average, all treatments	.....	.....	50	82

[1]All counts are averages of duplicate samples unless otherwise indicated. [2]Increased 22 per cent. [3]Single sample of 464 grams. [4]Labeled: "Active ingredients, chloramine-T 0.00016 and sodium benzoate 0.00032 by volume" when 68.3 grams are incorporated in 300 pounds of ice. [5]Increased 43 per cent. [6]Increased 120 per cent. [7]Single sample used for calculating count.

Freezing Market Shrimp: Samples of fresh shrimp, purchased from retail and wholesale dealers, were frozen in the laboratory and stored at —17.8°C.(0°F.). Bacterial counts were made immediately after the samples were received, after washing in water, after washing in bacteriostatic agents, after one day's frozen storage, and after two months' frozen storage (see results recorded Table 6). The bacteriostatic solution was prepared from a commercial preparation in the same concentration as would be present in germicidal ice made with this product.[3] The 46 per cent reduction produced by washing with water was in agreement with the 40

[3]Labeled: "Active ingredients, chloramine-T 0.00016 and sodium benzoate 0.00032 by volume" when 68.3 grams are incorporated in 300 pounds of ice.

TABLE 7

Bacterial Plate Counts on Commercially Frozen and Stored Shrimp

Sample[1] No.	In commercial frozen storage	Bacteria	Average of replicate samples	Organoleptic examination[2]
	mo.	*per gm.*		
147	3	750,000 1,200,000	1,000,000	Good
149	3	200,000 87,000	140,000	Good
150	3	170,000 180,000	180,000	Good
151	3	130,000[4]	.....	Good
152	3	270,000[4]	.....	Good
153	3	430,000[4]	.....	Good
154	3	700,000[4]	.....	Good
155	3	370,000 190,000	280,000	Good
156	3	370,000 280,000	320,000	Good
Average of 149-156 from a single commercial dealer, 310,000				
187	6	17,900,000 7,900,000	12,900,000	Poor[3]
189	6	23,500[4]	.....	Fair
191	6	160,000 120,000	140,000	Fair
193	6	24,000[4]	.....	Good
195	6	22,500[4]	.....	Good

[1] Each sample (or duplicate samples) from a five-pound box. [2] Immediately after thawing rated as: Excellent (appearance of freshly caught shrimp), Good (no pronounced odor or discoloration), Fair (pink or dark discoloration, some odor), Poor (marked odor and pronounced discoloration), Unmarketable. [3] Odor of amines present. Blackening along edges of shell segments, greenish-brown and pink discoloration evident. [4] Single samples used for calculating count.

per cent reduction previously reported, Green (1949b). Washing shrimp prior to freezing reduced the bacterial numbers, and two months' frozen storage of these samples produced a further 62 per cent reduction, thereby resulting in a product of considerably lower count than the untreated samples.

Commercially Frozen and Stored Shrimp: Bacterial counts on commercially frozen and stored shrimp from three different processors are recorded (Table 7). Samples 147 through 156 from a single commercial dealer represent nine five-pound boxes from a lot of 9,000 pounds seized

by the Federal Food and Drug Administration. Eight per cent of the entire lot was of questionable quality as judged by appearance and was discarded by the processor as not salable, and the remainder released. Samples 187 and 189, and Samples 191 and 193, were obtained from two different lots processed by another dealer. Samples 187 and 191 were of questionable appearance and would possibly have been refused by the buying public. There was no indication that appearance and deterioration, other than some dehydration, was due to frozen storage conditions, and it was concluded that insufficient care was used in segregating shrimp of initial poor quality prior to freezing. Certain chemical tests were reported on these samples by Fieger *et al.* (1947).

SUMMARY AND CONCLUSIONS

Bacterial counts were recorded on frozen shrimp of known pre-freezing history and on frozen shrimp from various commercial freezing plants after varying periods of storage at —17.8°C.(0°F.).

After 12 months' frozen storage, shrimp frozen on board the trawler immediately after catching, heading, and washing, contained 22,000 bacteria per gram. Headless shrimp held nine days in iced storage contained more than a million bacteria per gram after the same storage period. Samples of headless shrimp which were packed in the top layer of the icing bin, and samples headed and frozen after four days' iced storage as whole shrimp in the bottom of the bin, gave much lower counts during frozen storage than commercially frozen or laboratory-frozen samples of older pre-freezing ages. Lowest counts were found on shrimp which had been refrigerated without direct contact with ice before freezing.

An average reduction of 50 per cent was recorded on market shrimp after one day's frozen storage; this was increased to 82 per cent reduction by two months' storage. Percentage reductions produced by from two to 12 months' frozen storage of laboratory and commercially frozen products ranged from 48 to 99.2 per cent.

Bacterial counts on commercially frozen and stored shrimp varied widely and showed only a slight correlation with organoleptic examination.

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BACTERIOLOGY OF SHRIMP.[1]
IV. COLIFORM BACTERIA IN SHRIMP

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The sanitary significance of coliform bacteria in food warranted an investigation of freshly caught, iced, and frozen shrimp for the presence of this group of bacteria. If *Escherichia coli* is not a normal inhabitant of shrimp, the occurrence of this organism in appreciable numbers in market shrimp is indicative of faulty sanitation in handling the product. Frequency of occurrence of the species of the coli-aerogenes group in shrimp at the time they were caught, during iced storage, and when marketed as a fresh or frozen product is reported in this paper.

REVIEW OF LITERATURE

No previous reports on the occurrence of coliform bacteria in shrimp were found in the literature. Gibbons (1934) and Griffiths (1937) reviewed the literature on the occurrence of *E. coli* and related lactose-fermenting organisms in fish. A number of workers found that *E. coli* was not a normal inhabitant of the intestinal tract of fish, but was present along with other organisms of the coli-aerogenes group in fish taken from polluted waters, Fellers (1926), Gibbons (1934), Hunter (1920), Johnson (1904), and Zobell (1941). The presence of coliform bacteria, including typical *E. coli*, in iced market fish was noted by Fitzgerald and Conway (1937), Green (1920), Griffiths and Fuller (1936), and Nickerson, Fitzgerald, and Messer (1939).

EXPERIMENTAL PROCEDURE

During investigations on the bacteriology of shrimp, Green (1949a,b,c), all samples were examined for the presence of the coli-aerogenes group. Bacto-phenol red lactose broth was inoculated with one-ml. aliquots from all dilutions of each sample and incubated at 37°C.(99°F.) for 72 hours. The dilution in the mixing container was the lowest dilution used and averaged one to 5.5 in more than 400 samples. Presence of the coli-aerogenes group was verified by the procedures outlined in *Standard Methods for the Examination of Water and Sewage* (1936).

Cultures were purified by repeated streakings on Bacto eosin methylene blue agar and were identified according to Bergey, Breed, Murray, and Hitchens (1939) by a minimum of tests, as follows:

Aerobacter aerogenes: Imvic (indol, methyl red, Voges-Proskauer, citrate) −−++; gelatin not liquefied; glycerol fermented with gas production.

[1] This project was supported in part by a grant from The Refrigeration Research Foundation, Berkeley, California.

Aerobacter cloacae: Imvic + − − +; gelatin liquefied; glycerol not fermented with gas production.

Escherichia coli: Imvic + + − −.

Escherichia fruendii: Imvic − + − +; hydrogen sulfide produced.

Certain lactose-fermenters which were insufficiently identified, or which gave Imvic patterns not typical of the four species, were referred to as "unidentified" coliforms. Those cultures which produced gas in lactose within 14 days but not within 48 hours were referred to as "slow," and were identified as one of the four species by the tests mentioned above.

The coliform count per gram was determined from the highest dilution from which identified coliforms were isolated, i.e., if *E. coli* was only obtained from one ml. of the one to 5.5 dilution in the mixing container, the count was expressed as five coliforms per gram.

RESULTS AND DISCUSSION

Freshly Caught and Iced Shrimp: The total number of coliform bacteria and the species identified from freshly caught and iced shrimp are reported (Table 1). No coliform bacteria were found in sea water obtained 20 miles off-shore south of Barataria Bay, or 25 miles off-shore at Trinity Shoals Buoy. A sample of bottom mud from Trinity Shoals was also negative for this group of bacteria. The slime on freshly caught shrimp contained 210,000 bacteria per gram (Paper II of this series), approximately one-tenth of which were coliform bacteria of the genus *Aerobacter.* About one-half of all samples of freshly caught shrimp contained bacteria of the coli-aerogenes group, most of which were *Aerobacter* spp. *E. coli* was isolated from only one sample. These samples were examined within two hours after the net was pulled aboard; some were examined before the shrimp were washed or handled for sorting and heading. The data indicate that the greatest concentration of coliform bacteria was in or on the "heads" (cephalothorax), or slime, where the predominating species was *A. cloacae.*

Forty-two per cent of the iced and refrigerated samples contained bacteria of the coli-aerogenes group. Members of the genus *Escherichia* constituted only a small part of this group, whereas "slow" *A. cloacae* was present in 15 per cent of all samples examined, and was consistently isolated from whole shrimp and from the removed heads of iced whole shrimp. In following the same catch aboard the trawler, it was found that *A. cloacae* and "slow" *A. aerogenes* were present on the whole shrimp when caught; "slow" *A. aerogenes* was on washed samples after heading and before icing; and *A. aerogenes,* "slow" *A. cloacae,* and *E. coli* were found during nine days' iced storage in the bottom layer of the bin. In similar work with whole shrimp, only "slow" *A. cloacae* was isolated from freshly caught shrimp and from all subsequent samples during two days' iced storage in the bottom layer.

Forty-five per cent of the samples of freshly caught and iced shrimp examined before the trawlers were unloaded at the fish receiving house contained coliform bacteria. The average concentration from two boatloads was 75 coliform bacteria per gram. Members of the genus *Aerobacter* were isolated from 35 per cent of all samples examined. *E. coli*

TABLE 1

Coliform Bacteria in Fresh Shrimp

Description of sample	Number samples examined	Positive for coliforms	Average coliform count all samples	Per cent all samples containing the following species:						
				A. aerogenes		*A. cloacae*		*E. fruendii*	*E. coli*	Unidentified
				Typical	"Slow"	Typical	"Slow"			
		pct.	*per gm.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*
Bottom mud	1	0	0	0	0	0	0	0	0	0
Sea water	2	0	0	0	0	0	0	0	0	0
Slime from shrimp	1	100	2,700	0	100	100	100	0	0	0
Whole, freshly caught, unwashed	16	50	22	12	12	25	6	0	0	6
Headless, freshly caught, unwashed	7	71	110	14	14	29	29	14	0	29
Heads, freshly caught, unwashed	2	100	75	50	0	0	50	0	0	0
Whole, freshly caught, washed	3	33	27	0	0	0	0	0	33	0
Headless, freshly caught, washed	13	31	14	8	8	15	8	8	0	0
Total, freshly caught shrimp	41	49	38	12	12	20	12	5	2	7
Headless, refrigerated[1]	16	0	0	0	0	0	0	0	0	0
Headless, iced 1-10 days	32	56	79	9	0	3	16	6	12	28
Whole, iced 1-4 days	9	56	130	0	0	0	33	0	0	22
Heads from whole shrimp iced 1-4 days	1	100	1,100	0	0	0	100	0	0	....
Headless, from whole shrimp, iced 1-4 days	6	50	210	0	0	0	33	0	0	17
Total, iced and refrigerated shrimp	64	42	94	5	0	2	17	3	6	19
Total, freshly caught and iced shrimp	105	45	75	8	4	9	15	4	5	14
Composite from purchasing house	2	100	40	0	0	0	50	100	50	0
Spoiled, frozen	8	100	185,000	12	0	25	50	0	25	25

[1] Without direct contact with ice, one to seven days.

was present in only five per cent of the 105 samples, but was isolated from two samples composited in the purchasing house after the entire boatload was washed and handled by workers. Spoiled samples, selected in the purchasing house and frozen for subsequent bacteriological work, contained an average of 185,000 coliforms per gram after three days' frozen storage at —17.8°C.(0°F.). *E. coli* was present in two of the eight spoiled samples.

Headless shrimp, from which the shells had been peeled in preparation for processing in a wholesale house, had a total count of 280,000 bacteria per gram and a coliform count of eight per gram. *E. coli* was isolated from the sample. (This sample is not recorded in the tables.)

Market Shrimp: All 14 samples purchased from various wholesale and retail dealers contained coliform bacteria in an average concentration of 18,000 per gram (Table 2). Over one-half these samples contained *E. coli*. In the second paper of this series, it was reported that market shrimp contained from 600,000 to 129,000,000 bacteria per gram.

Frozen Shrimp: Data on members of the coli-aerogenes group isolated from frozen samples are given (Table 2). Thirteen of the market samples mentioned in the above paragraph were frozen and re-examined during frozen storage. At each examination, all samples were positive for coliform bacteria, and *E. coli* was present in eight of the 13 samples after two months' frozen storage.

Commercially frozen shrimp, examined after frozen storage for from two to 12 months, contained fewer coliform bacteria than the above-mentioned laboratory frozen samples of market shrimp. This was not unexpected, in view of the shorter pre-freezing iced storage and the lower total counts of commercially frozen samples (third paper of this series). *E. coli* was isolated from one five-pound carton after five, and again after seven, months of frozen storage, and from another carton from a different processor on the third month of storage. As in freshly caught and laboratory frozen market shrimp, members of the genus *Aerobacter* were isolated more frequently than *Escherichia;* and *A. cloacae* was the predominating species. Coliform bacteria (usually "slow" *A. cloacae*) were present after 12 months' frozen storage.

Laboratory frozen samples of known history (see Papers II and III of this series) contained fewer coliform bacteria than any commercially frozen shrimp examined. Only 14 per cent of the 62 laboratory frozen samples were positive for coliforms, and *E. coli* was not recovered from any sample.

It was interesting to note that the coli-aerogenes group was not isolated from fresh or frozen samples refrigerated without direct contact with ice before freezing. In previous papers of this series, it was reported that these samples also had the lowest total bacterial counts of all samples examined during iced and frozen storage.

SUMMARY

Forty-nine per cent of 41 samples of freshly caught un-iced shrimp contained members of the coli-aerogenes group of bacteria. With the exception of a single sample, in which *E. coli* only was found, all positive samples contained the genus *Aerobacter*, *A. cloacae* occurring more frequently than *A. aerogenes*.

TABLE 2

Coliform Bacteria in Frozen Shrimp

Description of sample	Number samples[1] examined	Positive for coliforms	Average coliform count all samples	Per cent all samples containing the following species:						
				A. aerogenes		*A. cloacae*		*E. freundii*	*E. coli*	Unidentified
				Typical	"Slow"	Typical	"Slow"			
		pct.	*per gm.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*
Market shrimp, before freezing	14	100	18,000	86	0	43	0	71	57	29
Market shrimp, 1 day's frozen storage	13	100	360	85	0	46	0	79	79	0
Market shrimp, 2 months' frozen storage	13	100	23	79	0	54	8	38	62	8
Commercially frozen samples (2-12 months' storage)	14	64	7	43	0	36	22	29	14	0
Total, frozen shrimp	40	80	120	60	0	40	12	40	40	2
Laboratory frozen samples (two to 12 months' frozen storage); of known pre-freezing history:										
Headless, iced 9-10 days, bottom layer	28	8	2	4	0	0	36	0	0	0
Headless, iced 9 days, top layer	4	100	2	25	25	0	50	0	0	0
Headless, iced whole 4 days, bottom layer	22	14	<1	0	0	9	0	0	0	5
Refrigerated 4-9 days without direct contact with ice	8	0	0	0	0	0	0	0	0	0
Total, laboratory samples	62	14	1	3	2	3	19	0	0	2
Total, all frozen samples	102	43	51	27	1	20	17	18	19	2

[1]Each sample is the average of duplicate or more samples from a single 300- to 400-gram box or five-pound carton.

Of 105 samples of freshly caught and iced shrimp examined on board the fishing vessels, 45 per cent was positive for coliform bacteria; 35 per cent of all samples contained *Aerobacter* spp., four per cent, *E. fruendii*, and five per cent, *E. coli*. The coli-aerogenes group was found more frequently in or on the "heads" (cephalothorax) and in the slime than in the headless shrimp (tails).

All of 14 samples of market shrimp examined contained coliform bacteria; 57 per cent of them contained *E. coli*. *E. coli* was found in 62 per cent of these samples after two months' frozen storage, and at this time all samples were positive for the coli-aerogenes group.

Only 14 per cent of 62 laboratory frozen and stored samples of known pre-freezing history contained coliform bacteria, and from none of these was *E. coli* isolated.

Since *E. coli* is rarely encountered in freshly caught shrimp, the occurrence of this organism in appreciable numbers in market shrimp is indicative of the need for more rigid sanitation in the handling of this product.

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BACTERIOLOGY OF SHRIMP.[1] V. EFFECT OF PEELING, GLAZING, AND STORAGE TEMPERATURE ON BACTERIA IN FROZEN SHRIMP

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The quality of frozen shrimp is known to be directly related to the care used in the pre-freezing selection, preparation, and packaging of the product, and to the promptness of freezing the shrimp after their preparation, Fieger and DuBois (1946), Fieger, Green, Lewis, and Kaloyereas (1947), Green (1949a). The pre-freezing preparation usually consists of heading and washing, and may include peeling, also. Use of peeled shrimp offers two advantages over the unpeeled product: it is more economical of storage space because the shells are eliminated, and consumer appeal is increased as a result of its table-ready condition. Glazing the shrimp offers another possible advantage, namely, longer storage life without dehydration or discoloration. Another factor of possible importance in determining quality of frozen shrimp, particularly after several months' storage, is the temperature employed for storage.

The purpose of the present investigation was to determine the effect of peeling, glazing, and storage temperature on the number of bacteria in frozen shrimp, and on the organoleptic quality of the product. This is a report of the bacteriological phase of the investigation.

METHODS

Headless shrimp, peeled and unpeeled, were washed in distilled water at 0°C.(32°F.) and packed in one-piece telescope cartons for freezing. One series of shrimp, which was not to be glazed, was overwrapped with glassine to prevent loss of moisture during storage. All shrimp were frozen at —40°C.(—40°F.). After 18 hours in the freezer, one series of shrimp was removed to a room at —12°C.(10°F.), the packages opened, and distilled water at about 0°C. added to cover the shrimp. The packages were returned to the freezer at —40°C. for 24 hours, then a portion of each lot (peeled and glazed, peeled unglazed, unpeeled glazed, and unpeeled unglazed) was placed at each of the following temperatures for storage: —12°C.(10°F.), fluctuating between —12 and —18°C.(10 and 0°F.), —18°C.(0°F.), and at —40°C.(—40°F.)

Bacteriological examinations were made immediately before freezing, four days after freezing, and after storage for one, two, three, four, six, nine, and 12 months. Methods employed were those described by Green (1949b), except that 10-gram (rather than 10-ml.) portions were taken from the blendor bowl to prepare the one to 10 dilution. All determinations were made with duplicate samples.

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RESULTS

In the determination of numbers of bacteria in shrimp it was observed that results obtained with duplicate samples occasionally varied 100 per cent, sometimes even more. These results were presumably due to marked differences in the bacterial load carried by individual shrimp, and the fluctuations might have been reduced by use of much larger samples. It was not possible, however, to use larger samples in the blendor bowls, consequently the average of counts obtained on two samples was employed. Because of fluctuations in counts between duplicate samples, changes in population of less than 50 per cent reduction, or 100 per cent increase, were considered of doubtful significance.

Bacterial counts made on shrimp immediately prior to freezing indicated considerably lower numbers were present in peeled shrimp than in the unpeeled product. Counts made four days after freezing at —40°C. (—40°F.) indicated apparent reductions of 82 per cent for the peeled shrimp and 40 per cent for the unpeeled.

TABLE 1

Bacteria in Frozen Headless Peeled Shrimp

Stored	—12°C.(10°F.)		—12 to —18°C. (10 to 0°F.)		—18°C.(0°F.)		—40°C.(—40°F.)	
	Glazed	Unglazed	Glazed	Unglazed	Glazed	Unglazed	Glazed	Unglazed
mo.	*per gm.*	*per gm.*	*per gm.*	*per gm.*	*per gm.*	*per gm.*	*per gm.*	*per gm.*
0	71,000[2]	66,000	71,000	66,000	71,000	66,000	71,000	66,000
1	44,000	51,000	79,000	56,000	120,000	70,000	59,000	73,000
2	90,000	.....[1]	72,000	75,000	130,000	72,000	280,000	29,500
3	65,000	130,000	.....[1]	.....[1]	230,000	73,000	.....[1]	.....[1]
4	72,000	71,000	260,000	81,000	270,000	90,000	400,000	81,000
6	180,000	40,500	44,500	55,000	310,000	100,000	180,000	74,000
9	23,000	32,000	420,000	83,000	520,000	52,000	45,000	60,000
12	34,000	27,000	120,000	43,000	450,000	100,000	250,000	110,000

[1] Determinations not made. [2] Each number is the average of two determinations.

The effect of the various storage temperatures and glazing on the survival of bacteria in peeled shrimp is shown (Table 1). Only at —12°C. (10°F.) was there pronounced reduction in numbers of bacteria after 12 months' storage. The reduction in numbers at —12 to —18°C.(10 to 0°F.) was slight and of doubtful significance. At all temperatures except the highest used (—12°C., 10°F.) glazing apparently exerted a protective effect on the bacteria. It was often observed that wider fluctuations between counts on duplicate samples (and between successive months) were encountered with glazed than with unglazed shrimp. Explanation for the fluctuations may be in uncontrollable variations in taking the glazed samples. Samples were thawed at room temperature just sufficiently to allow removal of individual shrimp. If the shrimp used for the count contained a considerable quantity of adherent glazing ice, the additional weight would tend to reduce the number of bacteria per gram, and thus introduce error. On the other hand, if all the adherent ice were removed from the shrimp, varying amounts of the surface slime of bacteria would also be removed, and thus influence the bacterial count obtained. Those shrimp

with the least disturbance of the natural slime layer and with the minimum of adherent ice would have the higher bacterial counts.

Effect of various storage temperatures on the bacteria in glazed and unglazed unpeeled shrimp is shown (Table 2). There was no significant decrease in numbers of bacteria in the shrimp except at —12°C.(10°F). Glazing was apparently without any appreciable effect on the unpeeled shrimp at any temperature used.

Coliforms were found in small numbers in a considerable proportion of the samples examined. During the period of storage the frequency of positive samples declined sharply at the highest temperature (—12°C., 10°F.), less rapidly at the intermediate temperatures, and apparently not at all at the lowest temperature employed (—40°C., —40°F.). Results are summarized (Table 3).

TABLE 2

Bacteria in Frozen Headless Unpeeled Shrimp

Stored	—12°C.(10°F.)		—12 to —18°C. (10 to 0°F.)		—18°C.(0°F.)		—40°C.(—40°F.)	
	Glazed	Unglazed	Glazed	Unglazed	Glazed	Unglazed	Glazed	Unglazed
mo.	*per gm.*	*per gm.*	*per gm.*	*per gm.*	*per gm.*	*per gm.*	*per gm.*	*per gm.*
0	440,000[2]	290,000	440,000	290,000	440,000	290,000	440,000	290,000
1	220,000	280,000	320,000	550,000	270,000	260,000	750,000	700,000
2	250,000	230,000	240,000	850,000	91,000	270,000	170,000	1,000,000
3	100,000	310,000	.....[1]	.....[1]	210,000	330,000	.....[1]	.....[1]
4	250,000	250,000	410,000	600,000	220,000	600,000	210,000	750,000
6	230,000	210,000	410,000	420,000	360,000	360,000	190,000	650,000
9	220,000	150,000	240,000	340,000	170,000	300,000	470,000	750,000
12	260,000	100,000	170,000	350,000	290,000	340,000	700,000	850,000

[1] Determinations not made. [2] Each number is the average of two determinations.

TABLE 3

Survival of Coliforms at Each Storage Temperature

Stored	—12°C. (10°F.)	—12 to —18°C. (10-0°F.)	—18°C. (0°F.)	—40°C. (—40°F.)	All temps.
mo.	*pct. pos.*	*pct. pos.*	*pct. pos.*	*pct. pos.*	*pct. pos.*
1	37[1]	63	100	75	69
2	13	75	50	100	60
3	63	....[2]	25	....[2]	....[2]
4	0	63	37	63	41
6	13	25	37	63	35
9	0	63	25	88	44
12	13	25	25	88	38
Pct. of all samples positive	20	52	50	80	....

[1] Each figure is based on eight samples: two peeled and glazed, two peeled unglazed, two unpeeled glazed, two unpeeled unglazed. [2] Determinations not made.

Of the coliforms isolated in the completed test and identified as to species, *Escherichia coli* was the most numerous, but was rarely found in large numbers in frozen shrimp. The most probable numbers of the coliform group and of *E. coli* found in the shrimp stored at the various temperatures are shown (Table 4). Many of the *E. coli* isolates were not typical in some characters: 29 per cent fermented cellobiose, 12 per cent did not produce indol, and 19 per cent failed to grow at 45°C.

TABLE 4

Coliforms in Frozen Headless Shrimp

MPN per Gram[3]

Stored	—12°C.(10°F.)		—12 to —18°C. (10 to 0°F.)		—18°C.(0°F.)		—40°C.(—40°F.)	
	Col.[1]	E.c.[2]	Col.	E.c.	Col.	E.c.	Col.	E.c.
Unpeeled Shrimp								
mo.								
1	0.13	0.13	1.62	0.63	2.32	2.19	11.36	2.25
2	0.12	0	1.76	1.63	0.14	0	120.56	1.32
3	0.26	0.14	.....	.....	0.13	0	.....	.....
4	0	0	1.25	1.25	0.22	0.12	15.14	1.25
6	0.13	0	0.14	0.14	1.25	1.25	1.45	1.45
9	0	0	1.62	0.14	0	0	11.77	11.49
12	0	0	0.14	0.14	0.25	0	1.68	0
Averages:	0.09	0.04	1.09	0.66	0.62	0.51	26.99	2.96
Peeled Shrimp								
mo.								
1	0.28	0	0.12	0	15.43	15.28	1.53	0.14
2	0	0	113.87	113.74	13.40	0.40	110.22	11.14
3	1.49	0	.....	.....	0.15	0.15	.....	.....
4	0	0	1.66	0.40	0.13	0.13	2.78	2.78
6	0	0	0.13	0.13	2.86	2.86	1.51	1.37
9	0	0	1.31	0	0.25	0.25	0.36	0
12	0.13	0	0.11	0.11	0	0	0.38	0.25
Averages:	0.27	0	19.53	19.06	4.60	2.73	19.47	2.61

[1] Col. = Coliforms. [2] E.c. = *Escherichia coli*. [3] Each figure is the average of four determinations (two glazed, two unglazed).

CONCLUSIONS

The number of viable bacteria in market shrimp was significantly reduced immediately after freezing at —40°C.(—40°F.). The reduction was greater in peeled than in unpeeled shrimp.

Storage at —12°C.(10°F.) was more destructive to the bacteria in shrimp than storage at lower temperatures. At —40°C.(—40°F.) there was no evidence of a decrease in numbers after storage for 12 months. No evidence was obtained that temperatures fluctuating between —12 and —18°C.(10 and 0°F.) were more lethal because of the fluctuations.

Peeled shrimp had lower bacterial counts than unpeeled.

Coliform bacteria were found in small numbers in 69 per cent of all samples examined after storage for one month. Storage death of coliforms was considerable at —12°C.(10°F.), less at the intermediate temperatures, and negligible at —40°C.(—40°F.), after storage for 12 months. *E. coli* was isolated from frozen shrimp more frequently than any other coliform organism.

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MICROBIOLOGY OF PAW-TSAY. I. LACTOBACILLI AND LACTIC ACID FERMENTATION

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Paw-tsay is a vegetable product processed and preserved by salting and guided fermentation of the juice, which is a process similar to the preparation of sauer-kraut and pickled vegetables in Europe and America. Various kinds of vegetables are made into paw-tsay. Vegetables most often used are turnip, red pepper, and cowpea. Method of preparation varies from house to house, although the essential features are similar. The essential features of paw-tsay making are:

(1) The vegetable, fresh or sun-dried, is submerged in boiled water solution of common salt. The strength of salt varies in most cases between four and six per cent. Pieces of whole vegetables or vegetable chips are packed and are in such proportion to the salt solution that the solution is only enough to fully cover the vegetable materials. Too little vegetable does not supply enough soluble substances for proper fermentation. Too much vegetable results in a certain amount of exposed portions, which often results in development of mold.

(2) Most often the materials are placed in earthenware vessels, specially made for the purpose (Fig. 1), or in other vessels that effectively

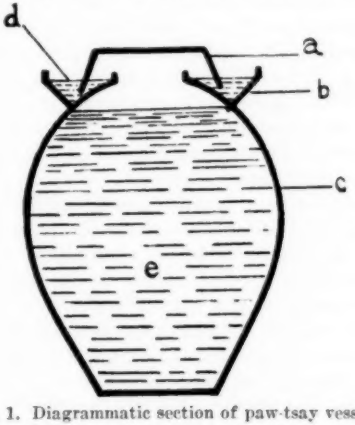

FIG. 1. Diagrammatic section of paw-tsay vessel.
a. Cover. b. Groove. c. Wall. d. Water-seal. e. Juice and vegetables.

prevent free aeration. The vessel is packed almost to its full capacity, so as to leave only a small air-space in it.

(3) The vessel is then kept at room temperature. Microbiological activities commence immediately, at a temperature of 15 to 20°C.(59 to 68°F.) or higher. The reaction of juice often changes from neutral to final pH value of four within 24 hours. Total acidity increases daily at the expense

of decrease of reducing sugar, which is extracted from the vegetables. Final total acidity is arrived at within one week, varying from 0.5 per cent to above one per cent acid calculated as lactic acid in sound products.

(4) Only two principal forms of microorganisms are observed to develop in sound paw-tsay, lactobacilli and yeasts. In high quality products, yeast is absent or in the minority. When the vessel has been opened too many times, or when it is not airtight, yeasts often develop into a film of mycoderma. The product under this condition is still edible, although less desirable. Only when microorganisms other than the lactobacilli and yeasts develop, forming a repulsive taste, or when the vegetables are rotten, is the product considered spoiled.

The present report describes the manner of lactic acid fermentation in paw-tsay made of turnips, and also the characteristics of the lactic acid bacteria isolated from the paw-tsay juice.

ISOLATION AND DESCRIPTION OF LACTOBACILLI

Microscopic examination of sound paw-tsay invariably revealed the gram-positive bacilli, arranged in chains, as the only or predominant form of bacteria. Forms of yeasts were often found in association with the bacteria. Ten strains of the bacteria were isolated. The morphological and cultural characters given in the following passages were based upon Strain 1006, unless otherwise mentioned. Other strains studied, however, conformed very closely with Strain 1006. Strain 1006, which was used in most of the experiments, was isolated from paw-tsay of turnip chips prepared October 3, 1947. This sample of paw-tsay was representative of the most sound product, in that it contained only the isolated lactobacillus, although it was prepared under conditions highly contaminating and had been opened many times. Strain 1006 was isolated October 16, 1947.

Morphological Characters. The bacteria were slender rods, 0.5 to 0.8 x 2 to 4 μ, arranged end to end in short chains, gram-positive, non-flagellate, non-capsulate, and non-spore-bearing.

Cultural Characters. The bacteria could not grow on nutrient gelatin medium (Difco), grew scantily or moderately on yeast extract glucose agar,[1] and grew moderately or heavily in yeast-extract glucose broth. The organisms were indifferent to oxygen supply, or facultatively anaerobic. Colonies developed in deep culture containing two per cent glucose were even from bottom to surface, respecting both size and number of colonies. Surface colonies on yeast-extract glucose agar were small, varying from pin-point colonies to circular colonies less than one mm. in diameter, with entire edge and convex elevation. Deep colonies were mostly bi-convex discs, also less than one mm. in diameter. The colonies were greyish-white and were buttery in consistency. Growth in liquid medium was, when young, cloudy, with neither surface growth nor sediments. In liquid cultures more than one week old, however, aggregates were formed at bottom. The bacteria were mesophilic, grew best at 20 to 30°C.(68 to 86°F.). Cultures in yeast-extract glucose medium kept at 50 to 55°C. (122 to 131°F.) gave only very slight turbidity.

[1] Yeast-extract glucose medium: Yeast-extract solution (10 per cent) 100 ml.; peptone five gm.; glucose 20 gm.; agar 15 gm.; water 1,000 ml. Mix. Tube. Autoclave. The agar is omitted for liquid medium.

Fermentation Tests. Eight strains of lactobacilli isolated from paw-tsay juice were taken for fermentation tests against glucose, sucrose, maltose, lactose, starch, arabinose, and mannitol. They produced acid but no gas (Table 1).

TABLE 1

Acid Production of Lactobacilli in Different Sugars[1]

No. of strains	Arabinose	Lactose	Mannitol	Sucrose	Glucose	Starch	Maltose
1003	1.09[2]	4.06	0.88	4.73	4.90	0.88	3.00
1006	4.68	4.23	4.59	4.22	5.15	1.26	3.53
1026	5.79	3.83	4.06	5.13	5.47	1.98	4.32
1032	3.35	3.79	4.02	4.03	4.85	2.11	3.88
1034	5.29	3.97	4.09	4.38	6.53	2.11	3.44
1035	4.09	4.59	4.06	5.20	5.47	1.85	3.80
1037	5.79	5.29	3.92	4.76	5.61	2.11	4.06
1050	5.64	4.23	3.67	5.29	5.82	2.02	3.93

[1] 25°C.(77°F.), seven days, in yeast-extract medium. [2] Acidity was determined by titration and calculated to mg. per ml. as lactic acid.

PAW-TSAY MADE BY PURE CULTURE OF LACTOBACILLUS

General characteristics of paw-tsay given in the introduction suggest that the lactobacillus as described in the above section is the principal microorganism involved in the biochemical changes in paw-tsay. The following passages describe an experiment carried out to see whether the lactobacillus alone is responsible for the proper fermentation of paw-tsay.

Five hundred grams of fresh turnip were dried in air to 60 gm. Three 15-gm. lots of dried turnip chips were placed in three sterilized flasks (No. 14, 15, and 16). They were then given the following treatments under aseptic conditions: 70 per cent ethyl alcohol, 0.1 per cent mercuric chlorid, and four changes of sterile water were added to the flasks, in the order given, shaken for five minutes each, and discarded. One hundred and fifty ml. sterilized salt solution containing five per cent salt was then added to each flask; the flasks were incubated four days. Flasks remained clear and no microorganism was found under microscope. Very weak bobbling was observed during this period, which was due to the respiration of the turnip chips. At the end of four days, Culture 1006 was inoculated into the flasks. Juice in the flasks became turbid the following day and typical paw-tsay fermentation set in in the normal manner.

The above experiment clearly shows that the lactobacillus alone is responsible for the normal fermentative activities of paw-tsay. Other microorganisms are either harmful or harmless contaminants.

PRODUCTION OF LACTIC ACID IN PAW-TSAY

In sound paw-tsay, increase of acidity accompanies the onset of turbidity. Acidity is produced at the expense of decreasing sugar, which is extracted from the vegetable materials. The turbidity remains for one week or more, and then the majority of microorganisms settle down as sediments, leaving the juice only slightly turbid. Time of sedimentation corresponds, roughly, to the arresting of acid production and the disappearance of reducing sugar.

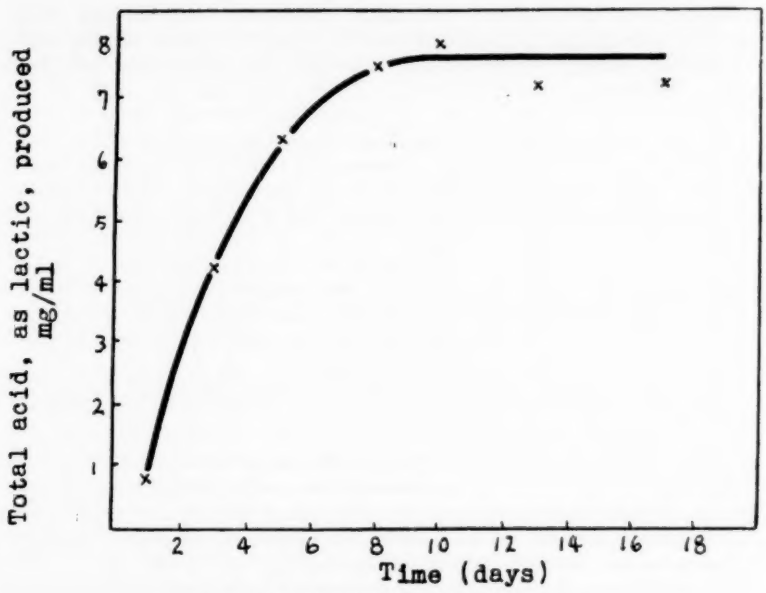

FIG. 2. The acid production in paw-tsay No. 7.

The manner of acid production typical to paw-tsay is shown (Fig. 2). Total acidity was determined periodically of an experimental sample (No. 7); 450 gm. turnip chips were air-dried to 150 gm. They were added to a vessel containing 600 ml. of six per cent salt solution and 25 ml. of shaw-shing-chu.[2] The preparation was incubated at room temperature. Ten-ml. lots were taken daily for the determination of total acidity. They were titrated against 0.1 N sodium hydroxide, using phenolphthalien as indicator. Total acidity reached a peak of 7.9 mg. acid as lactic acid per ml. juice on the 11th day. Yeast was found in the juice and gradually developed into a film of mycoderma, owing to repeated opening of the vessel.

The metabolic products varied from sample to sample, although lactic acid was always the chief end-product.

Total acidity, volatile acidity, and fixed acidity in three samples of paw-tsay (No. 19, 26, and 27) are given (Table 2). No artificial inoculation was made to Paw-tsay No. 19 and 26. Culture 1006 was inoculated to Paw-tsay No. 27. Paw-tsay No. 19 was already half a year old when determinations were carried out. Paw-tsay No. 26 and 27 were prepared May 25 and determined June 5 to 9, 1948. Only less than 10 per cent of total acidity was due to volatile acid in Paw-tsay No. 26 and 27. On the other hand volatile acidity in Paw-tsay No. 19 was more than one-third of total acidity.

[2] Shaw-shing-chu is a yellow wine which contains about 10 per cent alcohol. It is closely allied to the Japanese sake.

TABLE 2

Total Acidity, Volatile Acidity, and Fixed Acidity in Different Samples of Paw-tsay

No. samples of paw-tsay	Total acidity (calculated as lactic acid)	Volatile acidity (calculated as acetic acid)	Fixed acidity (lactic acid)
	mg./ml.	*mg./ml.*	*mg./ml.*
19	5.93	1.40	3.83
26	9.80	0.58	8.94
27	8.44	0.26	8.06

Permanganate distillation method [Birkinshaw (1931)[3]] was carried out to determine the lactic acid content of paw-tsay juices. In Paw-tsay No. 27 lactic acid found by this method was 105.1 per cent of fixed acid calculated as lactic acid. When the same method was conducted after removing volatile neutral substances from the juice, lactic acid found was 99.9 per cent of fixed acid. In Paw-tsay No. 26, lactic acid found by this method on whole juice was 92.5 per cent of fixed acid.

Two hundred and fifty ml. of juice were collected from Paw-tsay No. 14, 15, and 16, the paw-tsay made under bacteriologically controlled conditions, and its lactic acid isolated as zinc lactate.

LACTIC ACID PRODUCTION FROM GLUCOSE BY PURE CULTURE

Two 300-ml. Erlenmeyer flasks, each containing 250 ml. sterilized metabolic solution,[4] were inoculated with Culture 1006 and incubated at 25 to 30°C.(77 to 86°F.). Total acidity was determined by titration with 0.1 N NaOH, using phenolphthalien as indicator. Reducing sugar was determined by photometric method. (See Appendix.) Daily increase of total acidity and daily consumption of reducing sugar, which coincide with each other, are shown (Fig. 3).

A further study of lactic acid production by different strains of lactobacilli was made (Table 3). It was found that Strains 1006, 1026, 1032, 1034, 1035, 1037, and 1050 were homofermentative lactic acid bacteria, converting glucose utilized nearly quantitatively to lactic acid; and Strains 1003, 1051, and 1053 were heterofermentative lactic acid bacteria, converting about half the glucose utilized to lactic acid. All strains produced very small amounts of volatile acid (less than 20 mg. volatile acid, as acetic, per 100 ml. fermentation juice), and no gas.

YEAST AND OTHER CONTAMINANTS

Yeasts occur in most home-made paw-tsay. When aeration is not effectively prevented, due to drying of the water seal or too repeated lifting of cover, yeasts may grow offhand and develop into a thick film of mycoderma. This is considered as undesirable but by no means inedible. The "soundness" or "edibleness" of paw-tsay is very loosely defined. Objectionable flavor seems to be the only criterion. A partion of paw-tsay served in a restaurant has been found to contain, under microscope, instead of

[3] Birkinshaw, J. H., 1931. Biochem. J. 25, 1527.

[4] Metabolism solution: Glucose 20 gm.; $(NH_4)_2SO_4$ two gm.; KH_2PO_4 one gm.; $NaNO_3$ two gm.; $MgSO_4 \cdot 7H_2O$ 0.5 gm.; NaCl 50 gm.; $FeCl_3$ trace; yeast-extract solution (10 per cent) 100 ml.; water 1,000 ml.

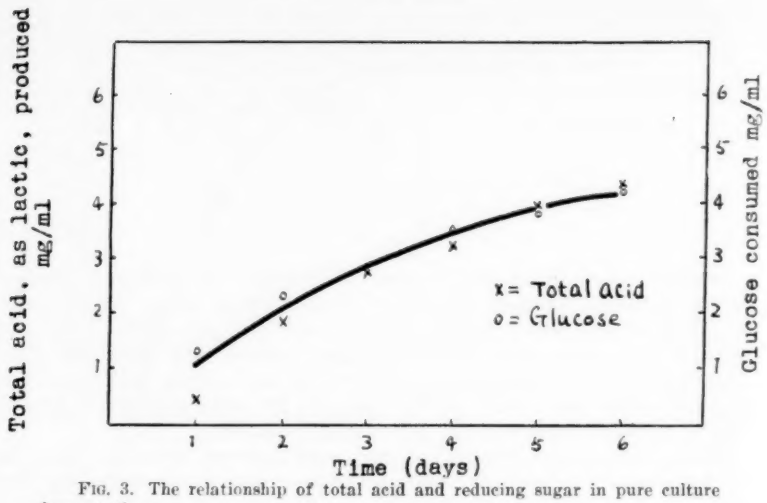

FIG. 3. The relationship of total acid and reducing sugar in pure culture fermentation.

the gram-positive lactobacilli and yeasts, chiefly gram-negative organisms of various shapes and sizes. This sample contained 12 per cent salt, and only 0.65 per cent acid, as lactic. It seemed that the restaurateur had resorted principally to the strength of salt to discourage microbiological activities. The product was really salt-preserved vegetables disguised as paw-tsay. The salting in this case was not strong enough, and consequently, various forms of microorganisms developed in it. Such product might have been a dangerous medium for spreading food-borne infectious diseases.

Experiments made in this laboratory included one lot of turnip chips soaked in 20 per cent salt solution. The lot remained, up to time of writing this report, free from microbiological activities of any description. Pickles of cucumber are usually preserved in 20 per cent brine. The amount of salt used to prepare paw-tsay, mostly from four to six per

TABLE 3

Lactic Acid Production From Glucose by Different Strains of Lactobacilli[1]

No. of strains	Sugar used	Total acid	Lactic acid	$\frac{\text{Lactic acid}}{\text{Sugar used}} \times 100$
	mg./ml.	*mg./ml.*	*mg./ml.*	
1003	10.00	5.39	5.59	58.9
1006	7.00	6.07	5.85	83.6
1026	7.60	6.50	6.20	81.6
1032	7.00	5.78	5.70	81.4
1034	7.75	6.24	6.49	83.5
1035	7.50	6.28	6.75	90.0
1037	7.00	5.77	5.93	84.6
1050	11.00	9.21	8.63	78.4
1051	8.10	3.69	3.90	48.2
1053	7.50	4.10	4.35	58.0

[1] 30°C.(86°F.), 10 days, yeast-extract medium.

cent, is not meant to prevent development of all forms of microorganisms. Its importance lies in selective retardation of undesirable microorganisms. Lactobacilli and yeasts grow freely in salt concentration of this range. Development of other microorganisms is, though not prevented, greatly retarded. Such condition permits the lactobacilli, and the yeasts, to establish a saturated mass concentration, and to produce enough acidity so as effectively to prevent the development of other microorganisms. In a sample of paw-tsay made without added salt in this laboratory, various forms of bacteria, yeasts, and molds were found to develop in the juice and also attack vegetable tissue.

Of the lactobacilli and the yeasts, which develop equally well at the usual salt concentration employed, lactobacilli are encouraged in properly made paw-tsay by restricted aeration, as discussed at the beginning of this section.

SUMMARY

A preliminary report on microbiology of paw-tsay is given.

Microbiological activities in paw-tsay are confined to juice only. Vegetable materials are not attacked.

Proper salt concentration and anaerobic conditions are necessary for the carrying on of successful fermentation.

The principal fermentation product of paw-tsay juice is lactic acid.

Strains of lactobacilli have been isolated and studied. Some strains are homofermentative lactobacilli. Others are heterofermentative lactobacilli.

Lactobacillus alone is responsible for typical fermentation of paw-tsay.

Yeast is a common contaminant, which, when not forming a thick film of mycoderma, is considered harmless.

ACKNOWLEDGMENT

The author wishes to thank Professor H. K. Chen, Head of the Department of Agricultural Chemistry, and Professor H. Zanyin Gaw, Department of Biology, for their kind suggestions and criticisms throughout this work; and the authorities of National Wu-Han University for the facilities supplied.

APPENDIX

SIMPLE PHOTOELECTRO-COLORIMETRIC METHOD FOR DETERMINING REDUCING SUGAR IN FERMENTATION JUICE

A simple photoelectro-colorimetric method has been devised for determination of reducing sugar in fermented vegetable juice. The method has approximately the same range and accuracy as the standard saccharometer method. The concentration of reducing sugar most conveniently handled by this method is one to 100 mg. per ml. of reducing sugar, which is 50 to 100 times more concentration than that of Folin-Wu's colorimetric method. The method takes advantage of the classical Fehling's reduction reaction by measuring the reduction of intensity of blue color. This is different from Folin-Wu's color reaction, in which the intensity of a yellow color is measured.

REAGENT AND APPARATUS

Photoelectric Colorimeter. A Lumetron photoelectric colorimeter, Model 400 GB.

Soxhlet Modification of Fehling Solutions. Prepare by mixing immediately before use equal volumes of (a) and (b).

(a) *Copper Sulfate Solution.* Dissolve 34.639 gm. of $CuSO_4 \cdot 5H_2O$ in water, dilute to 500 ml., and filter through prepared asbestos.

(b) *Alkaline Tartrate Solution.* Dissolve 173 gm. of Rochelle salt and 50 gm. of NaOH in water, dilute to 500 ml., allow to stand two days, and filter through prepared asbestos.

Standard Reducing Sugar Solution. Dissolve 10 gm. of pure glucose in 100 ml. of water and dilute to one liter in volumetric flask. Add a few crystals of thymol for preservation.

TEST PROCEDURE

Pipette five ml. exactly of Fehling Solution (a) and five ml. of Solution (b) in a 50-ml. volumetric flask. Place two ml. of fermentation juice, or aliquot containing about 10 to 40 mg. of reducing sugar, into the flask. Add water to the mark. Mix. Heat the solution over boiling water bath for about five minutes, centrifuge or filter through asbestos. Transfer about 10 ml. of the clear supernatant solution into optically-matched comparison tubes. Read per cent transmission in Lumetron photoelectric colorimeter, using red filter 650, against water as the blank set at 100 per cent transmission. The mg. of reducing sugar contained in unknown solution is obtained from the calibration graph. Calculate by the following formula:

$$\text{mg./ml. of reducing sugar in unknown solution} = \frac{\text{mg. of sugar found in graph}}{\text{ml. of unknown solution taken}}$$

CALIBRATION PROCEDURE

Pipette five ml. exactly of Fehling Solution (a) and five ml. of Solution (b) into each of 11 50-ml. volumetric flasks, and pipette 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, and 5 ml. of standard one per cent glucose solution into each flask. Add water to the mark. Mix. Heat the solution over boiling water bath for about five minutes. Centrifuge or filter through asbestos. Transfer about 10 ml. of clear supernatant solution into 11 optically-matched comparison tubes, and label.

Examine the comparison tubes in a Lumetron photoelectric colorimeter, using red filter 650. Distilled water is used as blank set at 100 per cent transmission. If the calibration is carried out properly, 40 mg. of reducing sugar should read somewhere near 70 per cent transmission.

Plot the results on a semi-log graph paper. An approximately straight line has been obtained with the range of 20 to 80 per cent transmission, or 10 to 40 mg. of reducing sugar in solution.

DETERMINATION OF EFFECT OF HEAT ON PEANUTS AND THE STABILITY OF THIAMINE IN ENRICHED PEANUT BUTTER

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Peanuts have been long considered a good source of protein, the minerals calcium, phosphorus, and iron, and vitamins of the B-complex, especially thiamine. This has been frequently assumed for roasted or cooked peanuts, as well as for the raw, and there is evidence that protein, riboflavin, and pantothenic acid do withstand the heat treatments quite well, Buss and Goddard (1948) and Dunn and Goddard (1948). However, some loss of thiamine is to be expected and has been reported in the literature. Pickett (1942) found 1.35 μg. of thiamine per gram of sample of peanut butter as compared to values of 10 μg. in raw peanuts. Lane, Johnson, and Williams (1942) found 0.94 μg. of thiamine per gram of cooked peanuts. Using a biological assay procedure, Miller, Louis, and Peterson (1943) reported 3.2 to 4.5 μg. of thiamine per gram in four samples of peanut butter.

Since the raw peanut is a good source of thiamine, more information is needed regarding the effect of processing upon this vitamin. Further, since the practice of fortifying foods has been extended to peanut butter, the stability of thiamine in enriched peanut butter has been investigated.

EXPERIMENTAL AND DISCUSSION

Among the considerations to be taken into account in discussing the thiamine content of raw peanuts, the source and type of peanuts should certainly be first. Two other considerations are method of extraction of the thiamine from the sample and method of assay.

Samples of Virginia runner and Spanish peanuts were obtained from 11 different sources which normally supply the trade. Digests of these samples were prepared by heating a portion of ground sample with 0.1N HCl for one hour in a boiling water bath and by further treating overnight with a buffered four per cent solution of a papain-takadiastase mixture. After filtering at pH 4.5 to remove protein, these solutions were assayed for thiamine by the thiochrome method as described in the Manual of the Association of Vitamin Chemists (1947).

Thiamine content of these Virginia runner and Spanish peanuts, from sources noted, is shown (Table 1).

Values found for Spanish peanuts agree within the range of the experimental error, with the exception of two samples. These two low values may be accounted for by local conditions under which the peanuts were grown. The runner peanuts show a somewhat wider range, yet an average of several lots agrees closely with the average of several lots of Spanish peanuts. Where two types come from the same locality the values agree also within the range of experimental error, ± five per cent.

These data indicate that no significant variation in thiamine content can be attributed to type or source of the raw peanuts studied.

TABLE 1

Effect of Variety and Source Upon Thiamine Content of Peanuts

Source	Thiamine in:	
	Spanish	Runner
	μg./gm.	μg./gm.
Virginia	.....	11.7
North Carolina	.....	12.2
Albany, Ga.	11.5	10.1
Durant, Okla.	9.8	.....
Blakely, Ga.	11.0	10.4
Stephensville, Tex.	10.1	.....
Leesburg, Ga.	11.5	12.1
Arlington, Ga.	10.2	9.7
Sylvester, Ga.	11.7	11.4
Donnalsonville, Ga.	11.4	.....
Colquitt, Ga.	9.6	.....
Averages	10.9	10.8

Values for raw peanuts are somewhat higher than those reported previously in the literature, but these variations may be accounted for by differences in hydrolysis and assay techniques.

Effect of Heat: In order to determine the effect of heat upon thiamine content, samples were obtained from five lots of peanuts roasted in a rotary radiant-heat type of oven at 155 to 160°C.(311 to 320°F.) for 20 minutes. This type of oven consists of flame-heated panels which reflect heat to a rotary drum containing the charge. After roasting, cooling is effected by forced draft.

In one lot, samples were withdrawn from the oven at five-minute intervals to determine rate of loss of thiamine. Three lots of roasted peanuts were marked, and samples of peanut butter manufactured from these were also obtained. Samples were extracted and assayed for thiamine by the procedure described for raw peanuts. Samples of peanut butter were dispersed in a Waring Blendor before aliquoting and extracting. Values obtained are reported (Table 2).

Loss of thiamine was not significant during the first five minutes of roasting, due to the fact that the roaster and the contents required some

TABLE 2

Effect of Heat Upon Thiamine Content of Peanuts

Description of sample [1]	I [2]	II [2]	III [2]	IV [2]	V [2]
	μg./gm.	μg./gm.	μg./gm.	μg./gm.	μg./gm.
Raw	11.56	12.3	12.0	12.5	13.6
Roasted 5 minutes	11.35	.....	.....	.....	.....
Roasted 10 minutes	10.75	.....	.....	.....	.....
Roasted 15 minutes	5.18	.....	.....	.....	.....
Roasted 20 minutes	2.32	.....	.....	.....	.....
Cooled	1.58	1.88	1.89	1.76	2.12
Blanched, ground, and manufactured into peanut butter	0.80	.....	0.70	0.63	.....
Pct. thiamine retained in peanut butter	7	.....	6	5	.....

[1] All peanuts were runner type, except Lot V, which were Spanish. [2] Composite samples of three to five bags of peanuts used to charge roaster.

time to rise to the temperature at which thiamine is destroyed. After five minutes, however, thiamine was destroyed rapidly, and after 20 minutes of roasting, followed by cooling, only 14 per cent of the original thiamine remained. Thiamine in peanut butter falls in the range of 0.4 to 1.3 μg. of thiamine per gram, reported in 13 samples by Higgins, Holley, Pickett, and Wheeler (1941).

Stability: The stability of thiamine in an enriched peanut butter was determined by storing samples under various conditions. Capped jars of peanut butter from the same batch were obtained on the day of production. One sample was assayed immediately to determine the original thiamine content. Several jars of product were stored at each of the following temperatures: —29, 4, 18, 27, 37, and 54°C. (—20, 40, 65, 80, 99, and 130°F.). The —29°C. samples served as controls, since it was expected no loss of thiamine would occur at that temperature; 4°C. was chosen to simulate commercial- or home-refrigerated storage. The 18 and 27°C. temperatures represented different conditions of warehouse, retail store, and home storage. This is also the prevailing temperature range for a large part of the year; 37°C. simulated extreme climatic conditions; 54°C. was chosen to subject the sample to a much more severe condition than any expected for a product like peanut butter, and was used only to accelerate storage losses. At intervals of one, two, three, and five months, one jar was withdrawn from storage at each temperature and assayed in duplicate for thiamine, using extraction and assay techniques described for other samples. Values obtained are shown (Table 3).

TABLE 3

Retention of Thiamine in Enriched Peanut Butter[1]

Temp.	Initial	1 month		2 months		3 months		5 months	
°F.	μg./gm.	μg./gm.	pct.	μg./gm.	pct.	μg./gm.	pct.	μg./gm.	pct.
—20	20.3	20.3	100	20.3	100	20.3	100	20.2	99.5
40	20.3	20.2	99.5	.....	.....	20.2	99.5	20.1	99.0
65	20.3	20.1	99.0	19.5	96.0	18.1	89.2	18.3	90.1
80	20.3	19.6	96.5	18.7	92.0	16.1	79.4	12.5	61.5
99	20.3	17.7	87.0	16.9	83.3	15.1	74.4	11.9	58.5
130	20.3	13.8	68.0	10.8	53.2	7.6	37.4	3.76	18.5

[1] Thiamine as μg. per gram and per cent retained.

By plotting per cent retention against time on semi-logarithmic paper a family of rectilinear curves can be obtained, indicating loss of thiamine occurs according to a first-order reaction. Hence, for any given temperature, a constant percentage of thiamine will be lost during successive time intervals. At 18, 27, 37, and 54°C. respective losses per month amount to two, seven, 11, and 28 per cent of actual thiamine contents. Losses at —29 and 4°C. were negligible.

The data indicate that enrichment of peanut butter is practicable, since under moderate storage conditions thiamine retention is excellent. Enrichment to the level of thiamine in raw peanuts makes peanut butter a good source of dietary thiamine.

SUMMARY

The thiamine content of Virginia runner and Spanish peanuts from 11 different sources was determined. No significant variation between the two types and sources was observed.

The thiamine content of raw and roasted peanuts was found to average 12.4 μg. per gram and 1.8 μg. per gram, respectively. Three lots of peanut butter showed an average of 0.71 μg. per gram.

Retention of thiamine in enriched peanut butter stored at —29, 4, 18, 27, 37, and 54°C. for periods of one to five months was determined. Thiamine losses of zero, zero, two, seven, 11, and 28 per cent per month were found at the respective storage temperatures.

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COMPOSITION AND NATURE OF APPLE PROTEIN[1]

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Little information is available regarding the composition and nature of the protein systems of fruits, and few methods have been developed for their separation and identification. This study was made to investigate the composition and characteristics of protein from apple tissue.

REVIEW OF THE LITERATURE

Among the few investigations reported on the nature of proteins of fruits is that of Smith (1925), who isolated a protein from the pulp of burred orange juice which differed from any of the conventional classes of proteins by its unusual solubility characteristics. Addition of acetic acid to filtered alkaline extracts produced a precipitate containing protein at a pH around 4.7. Since the pH of the orange juice was 4.3, it followed that under ordinary conditions the protein would be insoluble within the fruit. A polysaccharide resembling pectin accompanied the protein and appeared to be closely associated with it.

In the extensive studies on the nitrogen metabolism of apple fruits, Hulme (1935a and b, 1936a and b, 1937, 1938) and Hulme and Roach (1936) observed changes in nitrogen distribution in the fruit during varying phases of development and storage. In studies on the protein, or alcohol insoluble nitrogen fraction, Hulme (1939) reported that the protein of both fresh apple tissue and the residue left after extraction of the tissue with 85 per cent ethyl alcohol is very resistant to the usual protein solvents. Best results were obtained by extraction with dilute sodium hydroxide, which caused 82 per cent of the nitrogen of the tissue to go into solution. When the pH of the extract was adjusted to 3.5, a brownish-red precipitate was formed which represented 80 per cent of the nitrogen of the extract. This precipitate had a nitrogen content of 8.3 per cent, and gave characteristic tests for protein material. The protein was believed to be considerably degraded during the course of this procedure.

In further studies, the same author (1946a, b, and c) devised a method whereby approximately one-half the protein of apples could be extracted without denaturation. Extraction of frozen, ground tissue with warm borate buffer yielded an extract from which a precipitate representing 50 per cent of the protein nitrogen of the original tissue could be recovered by adjusting the pH to three. Prolonged extraction with alkaline buffers at low temperatures yielded extracts containing as much as 85 per cent of the original protein. Preparations from such extracts suggested that a fairly stable complex appeared to be involved. It was indicated that

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since cytolysis of the tissue and other less drastic methods for removal of the protein yielded only insignificant amounts of protein material, there is a good possibility that *in vivo* the cytoplasm of the cells of the fruit must be at a much higher pH than the vacuolar sap. The author based the success of the method for extraction by alkaline buffers on the fact that the acids of the sap are neutralized before contact between the protein and the sap occurs, thereby preventing coagulation of the protein on contact with the sap.

EXPERIMENTAL PROCEDURE

Baldwin apples from both 1946 and 1947 crops of orchards of the University of Massachusetts were used in the course of the investigation.

The fruit was peeled, trimmed, cored, and sliced in approximately one-quarter-inch slices. The prepared slices for studies on extraction of protein were immediately packaged in 400-gm. quantities in heat-sealed, cellophane bags, and placed in a freezer at —23°C.(—9°F.). Simultaneously, another lot of the prepared material was dehydrated for analytical studies. Surface darkening was very slight with both methods of handling.

Estimation of Protein Nitrogen: Protein nitrogen was determined according to a modification of the method of Vickery, Pucher, Wakeman, and Leavenworth (1946). Protein nitrogen was obtained by determining the total nitrogen of samples of dried tissue which had been thoroughly extracted, first with alcohol to remove carbohydrates and simple nitrogenous substances soluble in this solvent, and subsequently with hot water to remove a small residual quantity of soluble nitrogenous material. It is assumed this procedure extracts all nitrogenous material other than protein, and that all protein is converted by drying and by treatment with hot alcohol into the insoluble denatured condition. Tissue so prepared was used not only for nitrogen determinations but subsequently for amino acid analysis. On this basis, protein nitrogen accounted for an average of 58 per cent of the total nitrogen of apples sampled during the investigation, with no significant difference in proportions of protein nitrogen to total nitrogen in the samples of the two years.

Qualitative Tests for Detection of Protein: The common tests, including Millon's reaction, Xanthoproteic reaction, Glyoxylic Acid (Hopkins-Cole) reaction, the Biuret test, and Ninhydrin reaction, were applied to both fresh apple tissue and extracts of apple in various solvents, with only questionable results. Although in some instances color reactions were observed, they were of an indefinite nature, owing apparently to interference by other reactive materials. Similar results were reported by Hulme (1946c). Millon's reaction yielded a red-brown color which, in the presence of the normal coloration of the test material, could not be considered significant. The violet color of the Ninhydrin reaction was likewise masked, but could be detected. The Biuret test yielded the brick-red coloration sometimes associated with proteoses and peptones.

Extraction and Isolation of the Protein Nitrogen Fraction: Initial experiments on the extraction of the protein nitrogen fraction were conducted, using conventional procedures. Various solvents were employed on fresh, frozen, and dried apple tissue. From these studies it became apparent that alkaline solvents were the most effective, with the best

results obtained by extraction of frozen tissue. Under these conditions, over 90 per cent of the total nitrogen of the tissue could be recovered. Maceration of frozen tissue in a Waring Blendor with 10 ml. of extractant for each gram of tissue permitted recovery in the filtered extracts of 86 per cent of the protein nitrogen of the tissue when alkaline extractants were used. Sodium tetraborate in a concentration of 1.7 per cent (0.05M) was ultimately found to be the most satisfactory medium, although dilute sodium hydroxide solution was slightly more efficient. Extraction with water yielded 27.8 per cent of the protein nitrogen; 10 per cent sodium chloride solution gave 30.7 per cent; and 80 per cent ethyl alcohol yielded 6.5 per cent.

Although sodium hydroxide solution extracted slightly more of the protein nitrogen from the tissue, sodium tetraborate solution was preferred because of its buffer properties and lesser reactivity. Since the filtered extracts had a final pH value of 8.7, commonly accepted techniques of isoelectric precipitation were employed in initial studies. With these techniques, flocculation of the protein material occurred within the range of values between pH 3.9 and 2.9, being greatest at the lower values. Preparations obtained by these procedures showed nitrogen contents varying from 5.4 to 6.2 per cent, indicating presence of impurities which could only be separated from the protein with difficulty, and with degradation of the protein fraction.

Salting-out procedures and dialysis yielded only small quantities of protein materials, none of which were representative of the protein fraction. These methods were therefore discontinued in favor of foam fractionation which was highly satisfactory for the purposes of the present study. Techniques and apparatus were described in a preceding paper, Davis, Fellers, and Esselen (1949).

Composition of Protein Nitrogen Fraction: All analyses of isolated protein were made on preparations obtained by foam fractionation. Individual preparations from 1,600-gm. portions of frozen tissue were weighed to provide data on yield, analyzed for total nitrogen, and combined to provide a uniform sample for further studies. A composite of 10 preparations, components of which varied in nitrogen content from 8.43 to 8.54 per cent, with an average of 8.50 per cent, was obtained.

The recovered protein material was light red-brown in color, and similar in appearance to preparations obtained by isoelectric precipitation methods. Amino nitrogen, determined from acid hydrolysates by the Van Slyke method, amounted to 7.95 per cent, or 93.5 per cent of the total nitrogen. From the nitrogen content of the frozen tissue and of the extracts, estimates of both net recovery of protein nitrogen per weight of tissue, and efficiency of isolation from the extracts were obtained. Data on individual preparations indicated average recovery of 50 per cent of the protein nitrogen in the frozen tissues, and an efficiency of 61 per cent for the isolation procedure. A light-red Biuret test detected in exhausted extracts was considered indicative of either incomplete removal of protein or possible presence of polypeptides remaining in the solution.

Amino Acid Composition: The question of whether the material recovered was representative of the protein nitrogen fraction was considered.

If preparations were representative, it was considered reasonable that similar proportions of amino acids should be found in the isolated material and the estimated protein nitrogen fraction of whole apple tissue. Significant differences would indicate either losses of the particular amino acids in the isolation of the protein material or their presence in the tissue either free or in the form of soluble peptides. Differences of the latter type could be detected by analyses for such amino acids in samples of the alcohol-insoluble residue of the tissue.

Nature of the data required indicated the use of microbiological procedures for amino acid analyses. Procedures and microorganisms employed in amino acid determinations were selected on the basis of previous experience in analyses of other products, and preliminary studies with dried apple tissue. At least three separate determinations were employed in the assay of each amino acid, in order to obtain average values, and all determinations were made in duplicate. In some assays, different organisms were used in replicate determinations to observe correlation of values, and all replicate determinations of the same amino acid were made on different samples of hydrolysate to assure representative values.

Although previous experience had indicated reproducible results in analyses for 13 amino acids, experimental procedures in the present study were outlined for assay of the 15 amino acids believed to occur commonly in plants—leucine, isoleucine, valine, phenylalanine, tryptophan, histidine, arginine, threonine, methionine, lysine, aspartic acid, proline, glutamic acid, serine, and glycine.

Acid hydrolysis at 121°C.(248°F.) for five hours was used for the liberation of the amino acids, except cystine, tyrosine, and tryptophan, for which the procedure of Greenhut, Schweigert, and Elvehjem (1946), employing pancreatin and hog intestinal mucosa with shaking for 24 hours, was used. In all assays, simultaneous determinations were made on samples of both dried apple tissue and protein preparation.

Assays for leucine, isoleucine, phenylalanine, tryptophan, and glutamic acid were made, using *Lactobacillus arabinosus 17-5* as the test organism, according to the general procedure of Schweigert, McIntire, and Elvehjem (1944), with additional modifications found by Millares and Fellers (1948) to yield more reliable standard curves.

Histidine, arginine, threonine, methionine, and valine were determined, using *Streptococcus faecalis R*. Basal medium was that reported by Reisen, Schweigert, and Elvehjem (1946) for determination of methionine with this microorganism, except that pyridoxine, choline chloride, and i-inositol were omitted as not being indispensable for proper growth of the microorganisms as indicated by Millares and Fellers (1948).

Lysine, aspartic acid, serine, glycine, threonine, and proline were assayed, using *Leuconostoc mesenteroides P-60* according to the general technique of Dunn and co-workers (1944a and b) and employing the basal medium modified by Reisen, Schweigert, and Elvehjem (1946) with the same omissions noted previously in the medium for *S. faecalis R*.

No suitable methods were found for microbiological estimations of alanine, cystine, or tyrosine, and efforts to obtain values for these amino acids in the present investigation were finally abandoned.

Assay series were incubated at 37°C.(99°F.) for 72 hours, and growth response measured by titration of acid produced. Approximately 0.1 *N* sodium hydroxide was found to give suitable titration values, and bromothymol blue was used as the indicator, except in assays using *S. faecalis R.* For the latter determinations, thymol blue gave more satisfactory end points.

Results of these determinations (presented Table 1) show comparative percentages of the amino acids as constituents of the protein preparation, and of the protein nitrogen fraction of the whole apple tissue estimated on the basis of a protein of similar nitrogen content. Since the protein preparation showed a nitrogen content of 8.5 per cent, the protein fraction of the apple tissue was estimated from the percentage of alcohol-insoluble nitrogen in the tissue, multiplied by the factor 11.76, rather than the conventional factor of 6.25.

TABLE 1

Amino Acid Composition of Estimated Protein Fraction of Dried Apple Tissue and Isolated Protein Preparation

Amino acid	Dried apple tissue	Protein preparation
	per. N × 11.76[1]	*per.*
Leucine	5.2	5.5
Isoleucine	5.3	5.1
Valine	3.7	3.4
Phenylalanine	3.3	3.2
Tryptophan	0.0	0.0
Glutamic acid	6.1[2](4.7)[3]	4.5
Histidine	1.4	1.5
Arginine	3.5	3.5
Threonine	3.1	2.8
Methionine	1.7	1.6
Lysine	4.0	3.7
Aspartic acid	4.3[2](3.1)[3]	3.2
Serine	3.6	3.2
Glycine	2.9	2.9
Proline	4.2	3.8
Cystine	.....	1.1[4]

[1] Estimated on the basis of protein with nitrogen content of 8.50 per cent. [2] Includes amino acid in a form not combined in the protein fraction. [3] Determined from alcohol extracted tissue with nitrogen content of 1.20 per cent. [4] Estimated from total sulfur minus methionine sulfur.

Deviations from the mean values derived from replicate determinations varied from ± 2 to ± 5 per cent. Least reliable standard curves were obtained in determinations of serine and glycine, with greatest deviations likewise occurring in these determinations. Curves in these assays showed high zero points, and only gradual slopes.

Determinations for tryptophan showed a growth response equivalent only to amounts of this amino acid present in the enzymes used for hydrolysis, indicating absence of this amino acid in both apple tissue and isolated protein. Efficiency of hydrolysis was verified by determinations of tryptophan in proteins of known tryptophan content, and excellent recovery obtained. Absence of tryptophan in the apple flesh and in the protein preparations is not in agreement with the findings of Hulme, previously

cited, who reported at least a qualitative test for this amino acid in his preparations. Use of the Hopkins-Cole reaction for presence of the indole nucleus of tryptophan did not yield results which justified the inclusion of this amino acid in the composition of the fruit or protein.

With but two exceptions values for amino acids in the protein preparation and in the estimated protein fraction of the whole apple tissue were in close agreement. All comparative values, except for aspartic acid and glutamic acid, fell within the limits of precision found in replicate determinations, indicating a high degree of correlation. Differences in comparative values for aspartic acid and glutamic acid, however, were considered to be significant, since they amounted to $\pm$ 14.7 and $\pm$ 15.1 per cent, respectively, as opposed to maximum deviations from mean values in individual determinations of $\pm$ 3.4 per cent for aspartic acid and $\pm$ 2.8 per cent for glutamic acid.

The possibility that only these amino acids were lost by attrition in the preparation of the protein, appeared to be remote in view of the close correlation found in determinations of other amino acids. The more likely probability of the presence of these amino acids free, or in the form of simple peptides in apple tissue, was suggested by the fact that aspartic acid and glutamic acid are frequently found in these forms, or related compounds, in natural products. According to Hawk, Oser, and Summerson (1947), it is more than likely that the amide (asparagine), rather than aspartic acid, occurs in the protein molecule, but is rapidly hydrolyzed into aspartic acid and ammonia during the process of protein cleavage. Similarly, the greater part of the total glutamic acid found after hydrolysis of proteins is probably derived from glutamine. This could account for the discrepancies noted in the present study, since uncombined amino acids, amides, or peptides would have been extracted by the alcohol and water treatment of the tissue in the determination of protein nitrogen, and similarly would have remained in solution during isolation of the protein.

To verify this assumption, samples of alcohol-extracted apple tissue from determinations of protein nitrogen were also included in replicate determinations for assays of these amino acids. Results of these determinations are those shown in parentheses (Table 1). Comparison of these values reveals close agreement between amounts recovered in the alcohol-extracted tissue and in the protein preparation, indicating that while the greater proportions of these amino acids were present in the original tissue as part of the protein, significant amounts were likewise present not combined with the protein. While amino acids in this form would not be absolute components of the protein fraction, they might be of considerable importance in the synthesis of protein in the fruit.

Results of these assays gave good indication that the isolated preparation was representative of the protein nitrogen fraction of the apple tissue, and further indicated that, with the exception of aspartic acid and glutamic acid, practically the entire amounts of the amino acids were present in apple tissue combined as component parts of the protein. Amino acids for which values were obtained were calculated to constitute 49 per cent of the weight of the protein preparation, and accounted for 86.3 per cent of the amino nitrogen.

Elementary Mineral Composition of the Protein Preparation: In view of the relatively low nitrogen content of the protein preparation, it was believed that an analysis of certain of the mineral constituents would provide valuable information in regard to its constitution and the possible presence of impurities.

Three 0.5-gm. samples were incinerated at dull-red heat for estimation of total ash and analyses of ash constituents. Results of these determinations, calculated as percentages of the protein preparation, were as follows:

Total Ash	1.00 per cent
Phosphorus (as P_2O_5)	0.48 per cent
Iron (as Fe_2O_3)	0.37 per cent
Silica	0.16 per cent

The portion of the ash soluble in nitric acid was accounted for by phosphorus; while the remainder, most of which was soluble in dilute hydrochloric acid, was found to consist essentially of iron, and a small amount of silica which volatilized on treatment with hydrofluoric acid. Calcium was not detected.

Sulfur, determined by ignition of samples of the protein preparation in a Parr bomb and precipitation of the sulfur as barium sulfate, was found to be present in amount of 0.64 per cent. On the assumption that all of the sulfur was combined in the form of amino acid sulfur, this value was used to calculate the amount of cystine present in the preparation, since the value for methionine accounted for only slightly more than half this amount.

Absence of calcium, and the relatively low ash obtained from the material, eliminated to a considerable extent the possibility of presence of pectic acid as an impurity. If the iron in the material had been combined with pectic acid in the form of an iron salt, an appreciably greater percentage of ash would have been obtained.

CONSTITUTION AND NATURE OF THE PROTEIN PREPARATION

Identification of Component Groups: Since the general reactions of the isolated complex did not resemble those found with common protein classes, the nature of the complex was investigated by tests for specific protein types and for the identification of substances either associated with the protein or present as impurities.

The most obvious feature of the isolated protein, prepared either by foam fractionation or by direct isoelectric precipitation from extracts, was the presence of a relatively large fraction of non-nitrogenous material, red-brown in color, and relatively inert chemically, which could not be separated without some degradation of the nitrogenous portion. Practically all this material appeared to dissolve in hot, dilute sodium hydroxide, to yield a dark-brown solution. Its insolubility in six and 12 per cent hydrochloric acid was demonstrated by hydrolysis procedures, either by boiling or autoclaving, but apparently some peptization slowly occurred under these conditions to give a clear-orange to red-brown solution. The peptized portion could be reprecipitated from such a solution by addition of sodium hydroxide until the solution assumed a deep-brown color, followed by acidification.

A quantity of this material was prepared by treatment of a sample of the protein preparation with dilute sodium hydroxide, filtration of the resulting dark-brown solution from the insoluble portion, and reprecipitation with hydrochloric acid. The resulting red-brown precipitate was filtered, thoroughly washed, and a portion resuspended in hydrochloric acid. As noted previously, the material was insoluble in hydrochloric acid, but appeared to be peptized by continued boiling, to give a clear red-brown solution. The filtrate obtained after boiling did not reduce Fehling's solution; nor was furfural produced in the course of the treatment, indicating the absence of carbohydrates. Solubility tests in various solvents performed with other portions, indicated some solubility in acetone, resulting in an orange-brown solution. Alkaline solution of the material was rapidly changed to a colorless solution by hydrogen peroxide.

The relatively inert chemical nature of this material and its solubility properties agreed in general with those reported in the literature for the class of substances termed phlobaphenes. Neubert and Veldhuis (1944), in studies on clouding and sedimentation in clarified apple juice, detected a substance corresponding in properties to phlobaphene in the sediment obtained from Pectinol-clarified juice of Winesap apples which had been filtered and stored. The composition and properties of the material obtained in the present study closely resembled the findings of these authors, and determinations were carried out as suggested by their work. These determinations, following essentially the procedures of Muller and Zellner (1935) in studies on phlobaphenes from chestnuts, revealed the presence of phenolic compounds of the type attributed to this class of catechol tannins, and indicated that this portion of the non-nitrogenous material was probably a phlobaphene or a similar tannin-like compound. Analysis of an ash of this material showed it to consist of iron and silica, and indicated that probably all or most of the iron and silica found in the ash of the original preparation was associated with this substance.

Residues remaining after acid hydrolysis of samples of the preparation were considered to be mixtures of humin and phlobaphene material, since these residues, when washed free of acid, yielded notable amounts of phlobaphene on extraction with hot, dilute sodium hydroxide. However, even after extraction of the phlobaphene, a considerable amount of residue still remained, which indicated the possible presence of another associated substance, one which was capable of forming humin, since the relative amount of humin was considered to be in excess of that which might be expected from condensation of susceptible amino acids and possible presence of nucleic acid.

Investigation of the nitrogenous fraction was directed toward identification of particular protein groups suggested by the composition and source of the material. Presence of phosphorus in the material, apparently all associated with the nitrogenous portion, suggested the possibility of the presence of nucleoprotein. Tests conducted according to the general procedure outlined by Hawk, Oser, and Summerson (1947) showed the presence of organically combined phosphorus, purine bases, and pentoses in the nitrogen fraction, indicating a portion of the preparation was nucleoprotein. Demonstration of the presence of purine bases in the material

indicated that some of the alcohol-insoluble nitrogen was purine nitrogen tied up in this form.

In the preparation of the protein fraction a distinct darkening of freshly collected foam, suggestive of an oxidative reaction, brought up the possibility that this reaction may have resulted from the presence of oxidative enzymes in the protein system. Although foam fractionation is known to cause some surface denaturation and coagulation of proteins, a darkening of this type, if enzymatic in nature, would indicate the presence of enzymes which were not affected by this foaming procedure, and which, therefore, could be demonstrated by qualitative tests.

For these tests, a small sample of freshly collected foam was allowed to settle, then centrifuged to obtain maximum collection of suspended material. The opaque supernatant liquid was decanted and saved, and the precipitate washed three times in the borate buffer solution to remove any of the liquid portion which may still have remained occluded in the protein precipitate. After final centrifuging, the precipitate was once more suspended in buffer solution, and samples of this suspension, of the original decantation, and of the clear solution of the extract which remained after foaming, taken for enzyme tests.

Samples were adjusted with a few drops of acetic acid to pH 5.5, reported as one of the pH optima by Sumner and Somers (1947), and 10-ml. portions of each tested with three per cent hydrogen peroxide for presence of catalase. Control samples, which had been boiled for 20 minutes, were also tested. Evolution of gas occurred quite strongly in the sample of the original decantation, and to a lesser extent in the extract remaining after foaming. Little activity was noted in the sample of the precipitate, and control samples were all negative.

To each of these samples was then added approximately one ml. of a fresh one per cent guiacol solution, after which the tubes were placed in a water bath at 30°C.(86°F) for approximately 15 minutes. Addition of acetic acid in the adjustment of the pH had changed the colored solutions to a light yellow, so reactions of the guiacol were readily observable. A definite brown coloration developed in the sample of the original decantation, and a slight coloration was observed in the sample of foam-exhausted extract. No color developed in suspensions of the precipitate or in the controls.

Results of these tests indicated that, while the oxidase enzymes were probably not quantitatively removed from solution by foaming, considerable quantities were concentrated and carried over in the foam, and remained uncoagulated in this fraction. While subsequent treatment unquestionably inactivated or coagulated these enzymes, the tests indicated their presence as constituents of the protein preparation.

Discussion of Possible Significance of Component Groups: In any investigation of protein materials, the identity of isolated preparations in respect to the natural complex existing in the tissues is always open to some question. In many instances, variations in composition will result from different methods of extraction and preparation, and with no criterion of identity or purity, conclusions must of necessity be based on the particular preparation obtained.

Of considerable concern in the present study was the low nitrogen content of the preparations, apparently resulting from presence of a relatively large non-nitrogenous fraction which seemed to be closely associated with the protein complex. Hulme, in his studies previously cited, also obtained preparations of apple protein of low nitrogen content, and of similar appearance and character, but made no effort to identify the associated substance or substances. Presence of such material in protein preparations of this type would lend credence to the assumption that a close relationship exists between it and the nitrogenous portion, but would not necessarily indicate it exists as an integral part of the protein, i.e., as a prosthetic group.

Characterization of at least some of this closely associated material as a tannin compound is believed to be of considerable significance in the present study, in connection with the unusual solubility characteristics of the protein. Since tannins form insoluble precipitates with certain proteins, it is not inconceivable that this occurs when the cells of the apple tissue are ruptured. The resulting environment would be highly conducive to a reaction of this type, and the greater part of the protein would thus be rendered insoluble. Solubility of the tannin material in alkali may be the reason for the increased solubility of the protein in alkaline extractants, rather than the possible neutralizing effect of the alkali on the vacuolar sap as previously proposed.

Presence of tannin closely associated with the iron enzymes of the oxidative system, which appear to be part of the protein fraction, could be of some practical significance. Tannin materials are known to be intimately associated with the enzymatic browning of apple and other plant tissues, and may possibly serve as substrates for the oxidases. A more thorough understanding of this relationship might indicate better methods for control of this process in cut-plant tissues.

An explanation of the possible presence of a pentose-yielding substance other than nucleic acid is indicated by the studies of Chibnall (1939) and others. These workers suggest that plant protoplasm is an "intermeshed pentosan-protein colloid," and that pentosans extracted along with the protein appear to follow the protein in and out of solution, making separation difficult. It is very possible that some of the pentose-yielding substance found in the preparation might be a combined pentosan of this type, in addition to nucleic acid which could be expected only in very small amount. This was indicated by the relatively large quantity of humin-like material remaining after acid hydrolysis of the protein. Humin is known to arise from polymerization of furfural formed during breakdown of pentosans, and also from its condensation products formed during destruction of liberated cystine, methionine, tyrosine, tryptophan, arginine, and histidine. The major portion of these amino acids, particularly cystine, tryptophan, and tyrosine, lost during acid hydrolysis of protein, may be accounted for by the formation of humin. The quantities of these amino acids detected in the preparation, even in combination with the amount of nucleic acid which could be expected as a constituent of the nucleoprotein, may not have been sufficient to yield the relatively large amount of humin obtained.

SUMMARY

Preparations were made of protein material isolated from Baldwin apples. Extraction of frozen tissue with 1.7 per cent sodium tetraborate solution, by maceration in a Waring Blendor, permitted recovery in the extract of 86 per cent of the protein nitrogen. Separation of the protein fraction by foam fractionation of extracts was found to be a highly satisfactory method.

The protein fraction of apple tissue appeared to be a complex of unusual solubility characteristics, made up of a number of protein systems. The isolated protein material, which was red-brown in color, had a nitrogen content of 8.5 per cent. Comparative amino acid analyses by microbiological methods indicated that preparations were representative of the alcohol-insoluble nitrogen fraction of the apple tissue.

Amino nitrogen amounted to 93.5 per cent of the total nitrogen of the isolated protein, and the 15 amino acids for which values could be obtained accounted for 86.3 per cent of this amount. These amino acids were calculated to constitute 49 per cent of the weight of the preparation. Notable amounts of aspartic acid and glutamic acid, over and above amounts combined in the protein, appeared to exist free or in the form of simple peptides or amides in the apple tissue. Tryptophan could not be detected in either the preparations or the tissue itself.

There was evidence that nucleoprotein constituted a part of the isolated complex, since analysis revealed the combined presence of phosphorus, pentose, and purine base radicals associated with the amino nitrogen.

A portion of the isolated complex exhibited definite enzyme activity, and showed typical oxidase reactions.

The low nitrogen content of the protein preparations was attributed to the presence of a relatively large amount of non-nitrogenous material which could not be separated without some degradation of the nitrogenous portion. This material was deep red-brown in color, relatively inert chemically, and appeared to be the agent which imparted color to the preparations. Its composition and general properties indicated it was phlobaphene or a very similar tannin-like compound. It was believed that formation of a stable, water-insoluble protein-tannin complex, very likely produced immediately upon rupture of the cells, may be responsible for the unusual solubility characteristics of the protein. There was also evidence that some closely associated protoplasm material may likewise have accompanied the protein in the preparations.

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ACCEPTANCE OF FRESH, FROZEN, AND CANNED ORANGE JUICES

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A spirited and extensive advertising campaign has recently introduced to the consumer market a new orange product—a semi-concentrated frozen juice that is claimed to taste like fresh juice, Anon. (1947). Inasmuch as canned orange juice has always fallen far short of this goal, because of undesirable off-flavors developed in processing and storage, Anon. (1948) and Henry and Clifcorn (1948), it is expected by the makers that the new frozen product will meet with widespread approval. Investigation of consumer acceptance of frozen and of canned orange juice seems indicated.

The present report deals with a study of the relative acceptance of fresh, two semi-concentrated frozen, and six canned orange juices among members of the Department of Food Technology, Massachusetts Institute of Technology. The problem fell naturally into two parts: (1) A preliminary study of market varieties of canned orange juice, to determine whether there was significant preference among brands and, if so, to select the most preferable brand or brands for further comparisons; and (2) determination of the relative acceptance of frozen, canned, and fresh orange juices. The results may be expected to give some indication of general consumer acceptance of the new product.

EXPERIMENTAL PROCEDURE

Experimental Design: A "paired-comparison" procedure, Hening (1948), was used to measure the relative acceptance of the juices tested. In this type of test the samples are presented to the judges in a series of pairs, each sample being paired in turn with every other sample in the group. Because the judge is asked to consider only two samples at one time, and required merely to decide his general preference between the two, this form of test lends itself well to use with untrained judges. In the present study, two pairs representing four different juices were given each day. Judges were asked to state which member of each pair was preferred or whether no preference existed and to append any descriptive remarks.

Judges: Sixty-five staff members and students (47 M, 18 F) of the Department of Food Technology served as judges. No demand was made for regular attendance; each judge presented himself at whatever hours and on whichever days were convenient. Daily attendance ranged from 43 to 55 and averaged 50 persons. Judges were given oral explanation of the object of the study and the general procedure. They were instructed to taste the juices in any order, repeating as necessary and taking water and resting between tastes until a decision was reached. Preferences were recorded in writing on individual ballots.

Selection of Juices: The study was arbitrarily restricted to early-season Florida Valencia orange juice. It was conducted during November and

December, a "between" season, when California oranges are leaving the market and Florida oranges are beginning to appear. The early-season Florida fruit yields a juice that is comparatively thin and watery and lacks the acidity and sweetness of juice from more mature, later-season fruit. The well-known seasonal variation in canned citrus juice is probably due to the fact that many packers utilize this early-season juice to a large extent. Unfortunately, in purchasing the products on the retail market there is no sure way of distinguishing newly-canned, early-season juice from the previous year's late-season pack. An attempt was made to control this variable by purchasing the canned and the frozen juices in large supermarkets where, it was hoped, the rapid turnover of stock would insure the obtaining of representative samples of the new pack in each case. Subsequent observations gave reasonable assurance of success in this respect.

In the first part of the study, six brands of canned, unsweetened Florida juice were compared. These were arbitrarily selected to represent a range in retail price (Table 1).

TABLE 1

Retail Prices of Canned, Unsweetened, Florida Orange Juice

Brand	Place of purchase	Price per can[1]	Price per ounce
		cts.	*cts.*
A	Specialty food store	44.0	0.95
B	Specialty food store	39.0	0.84
C	Small market	12.5	0.69
D	Small market	29.0	0.63
E	Supermarket	23.0	0.50
F	Supermarket	23.0	0.50

[1] Brand C was purchased in No. 2 cans (18 oz.); all other brands were purchased in No. 3 cans (46 oz.).

In the second part of the study, medium-sized fresh oranges were used. These were early-season Florida Valencias, purchased in bulk at a local supermarket. They retailed at eight pounds for 49 cents (price per ounce of juice calculated as approximately one cent). This juice was called Brand G.

Two widely-advertised brands of semi-concentrated, frozen Florida juice were also used in the second series. Both brands come in six-ounce cans, contents of which, when diluted as directed with three parts of water, yield 24 ounces of juice. Brand H sold for 25 cents and Brand I for 23 cents per can, or the equivalent, respectively, of one and 0.95 cent per ounce of juice of ordinary strength.

Storage of Juices: The first group of juices (canned) was tested over a period of 16 days. During this time the cans were held on open shelves at room temperature, duplicating retail market conditions. Cans were opened each morning, as required for the day's samples. Contents of the cans of a given brand were mixed and poured into a glass bottle, from which the juice was served during the day. Juice left at the end of the day was discarded, and new cans were opened the next morning. The second group of juices (fresh, frozen, and canned) was tested over a period of 10 days. For this second series new supplies of the selected canned

juices were purchased and stored under conditions similar to those for the first series. The fresh oranges were held in a cold room at 4°C.(40°F.). Juice was extracted for each day's samples on the previous afternoon, strained into glass bottles, and held overnight in the cold room. The frozen juices were stored at —18°C.(0°F.). Before use, they were mixed according to directions with three parts of tap water and poured into glass bottles. All juices were brought to room temperature in the morning before they were served.

Serving of Juices: Juices were presented to the judges in one-ounce portions, served in 150-ml. beakers. The latter were coded to prevent identification. Samples were usually judged immediately after they were poured, but in no instance stood for longer than 15 minutes.

RESULTS

The judges' preferences for the various brands of orange juice presented in the two series of pairings are shown (Table 2).

Preferences—Series I: On the basis of the judges' preferences (without reference, for the moment, to their degree of significance), the six brands of canned orange juice compared in Series I may be ranged in order of decreasing preference as follows: A, B, C-F, D, E. As the judges

TABLE 2

Judges' Preferences for Brands of Fresh, Frozen, and Canned Unsweetened Florida Orange Juices, Presented in Two Series of Pairings

Brand pairs	Judges' preferences	Significance of preferences[1]
	Series I	
A : E	47 : 4	**
B : F	44 : 4	**
A : C	38 : 10	**
B : D	39 : 10	**
C : D	33 : 17	*
E : F	20 : 31	....
B : C	38 : 8	**
D : E	31 : 15	*
A : B	28 : 26	....
C : E	39 : 14	**
A : D	35 : 4	**
C : F	25 : 25	....
B : E	43 : 4	**
D : F	19 : 25	....
A : F	31 : 13	**
	Series II	
A : G	5 : 46	**
H : I	43 : 7	**
A : H	1 : 54	**
B : G	16 : 37	**
A : B	4 : 44	**
G : I	28 : 20	....
A : I	6 : 43	**
B : H	4 : 46	**
B : I	2 : 41	**
G : H	24 : 16	....

[1] * Significant, P = 0.05; ** highly significant, P = 0.01. (Snedecor, 1946.)

were almost equally divided between Juices A and B, a more practical order would be A-B, C-F, D, E. This order is found to vary almost directly with price, Juice F being a notable exception.

Significance of Preferences—Series I: In tests involving brands at the upper end of the scale (A and B), the preferences are in general highly significant ($P = 0.01$). In tests involving brands at the lower end of the scale, the preferences are not in all cases significant, even at the $P = 0.05$ level. There are apparent some contradictions in preference among the lower brands. These may be the result of the greater difficulty experienced by judges when dealing with samples of poor quality, but are more probably due to existence of only slight variations in acceptability between the lower quality brands, with consequent overlapping of the confidence limits.

Position of Brands A and B at the head of the list is clearly established, inasmuch as the preferences expressed for each of these, when compared in turn with Brands C, D, E, and F are in every case highly significant ($P = 0.01$). The slight preference shown for Brand A when compared with Brand B (28:26) is not of significance ($P = 0.05$). As these two juices are widely different in character, Brand A being tart and Brand B sweet, it would seem the judges are about evenly divided in their liking for these two types of juice.

On the basis of results of this preliminary test, Brands A and B were selected for comparison with frozen and fresh juices in the second series.

Preferences—Series II: In accordance with preferences expressed, the five juices compared in Series II may be ranged in order of decreasing preference as G, H, I, B, A, or fresh, frozen, canned.

Significance of Preferences—Series II: The fresh juice (Brand G) was slightly preferred to each of the frozen juices, but this preference is not of significance ($P = 0.05$). In all other instances preferences expressed are highly significant ($P = 0.01$). It is evident, then, that the two canned juices, A and B, were significantly less preferable than the fresh juice and the two semi-concentrated frozen juices. Of the two frozen juices, Brand H was significantly preferred to Brand I, although no significant preference was indicated when each brand was compared with the fresh juice. Overlapping of the confidence limits is again evident.

In this second series a definite preference was expressed for Juice B over Juice A, whereas in the first series these two juices were judged equally desirable.

Explanation for the discrepancy probably lies in seasonal variation, since supplies of these juices for the two tests were purchased some three to four weeks apart and undoubtedly were drawn from different wholesale lots.

Judges' Comments: No formal analysis of the random remarks of untrained judges is possible, but some general description is interesting. Comments indicated a great variation in impressions and in personal likes and dislikes, the same juice being described as "sweet," "very sweet," "flat," "tasteless," and "sour" by various individuals. It was evident also that impressions formed by many of the judges were largely a matter of comparison. For example, Juice A, which was characteristically tart, was

described as "acid," "bitter," and "sour" when compared with Juice B (sweet), but as "sweeter," "like natural juice," and "having a nice orange tang" when compared with Juice E (off-flavor). The canned Juices A and B, when paired in Series I with canned juices of inferior quality, were described as "natural," "like fresh," "very good;" in Series II, when paired with fresh and frozen juices, they were described as "bitter," "sour," "acid," "sharp," and "lacking in flavor."

SUMMARY AND CONCLUSIONS

A paired-comparison procedure was used to determine preferences of some 50 judges for canned, frozen, and fresh early-season Florida Valencia orange juices. A freshly extracted Valencia orange juice, two high-priced brands of canned orange juice selected in a preliminary study as the most preferable of six common market brands, and two widely advertised, semi-concentrated, frozen juices were used in the study.

Within the limitations of the conditions outlined, the following conclusions seem warranted:

There is significant preference between market brands of canned, unsweetened, Florida orange juice. For the six brands tested, preference tends to vary directly with price.

The two high-quality, canned Florida orange juices selected are significantly less preferable than either fresh juice or the two frozen juices tested.

There is no significant preference between fresh, unsweetened, Florida orange juice and the two brands of frozen juice studied.

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COMPARISON OF COLIFORM AND ENTEROCOCCUS ORGANISMS AS INDICES OF POLLUTION IN FROZEN FOODS[1]

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In 1893, Theobald Smith, in referring to *Escherichia coli* stated that, "It is safe to infer that any organism so uniformly present in the intestinal tract . . . . really belongs there, and that its presence outside the intestines in soil and in water may be regarded as due to their contamination with fecal discharges of men or animals," Smith (1893). Such a statement might apply equally well to the enterococci, the streptococci of the human and animal intestine, Burke-Gaffney (1932).

The coliform group presents a problem in sanitation studies, because this group includes not only the fecal *E. coli*, which is a recognized indicator of pollution [Levine (1916), Parr (1939), and Tonney and Noble (1932)], but also *Aerobacter aerogenes* and intermediate types, which, although they occur in fecal material, have their natural habitat in soil and vegetable material, Gray (1932), Griffin (1940), and Tonney and Noble (1931b). However, it is now generally accepted that all members of the coliform group must be considered as indicators of fecal contamination because of their universal occurrence in the intestinal tract, Bardsley (1934), Kline (1935), and Parr (1938a and c).

On the other hand, organisms belonging to the enterococcus group have been definitely established as of intestinal origin [Broadhurst (1915), Ostrolenk and Hunter (1946), and Sherman (1937)], and their occurrence outside the animal body is attributed to survival rather than to growth.

In considering the two groups of organisms as indices of fecal contamination, it is important next to compare their respective abilities to survive in foreign environments and to tolerate adverse temperatures. A number of investigators including Lochhead and Jones (1936), Parr (1936 and 1938b), and Tonney and Noble (1931a), have demonstrated that the coliform organisms in fecal material, in culture, or in frozen foods, show a decrease in numbers during initial freezing and in the first few weeks of storage. In contrast, the enterococci have been reported to be exceedingly resistant to low temperatures and to adverse conditions, Dible (1921), Evans and Chinn (1947), and Sherman (1937). Comparative studies by Ostrolenk and Hunter (1946) and Ostrolenk, Kramer, and Cleverdon (1947), with both groups of organisms, have revealed that in feces, contaminated soil, or foods, fecal streptococci soon outnumber the coliform organisms which are initially predominant but which are less hardy. It has, therefore, been suggested that the enterococci might prove superior to the coliform organisms as an index of fecal contamination in frozen foods.

[1] Contribution No. 282, Division of Bacteriology and Dairy Research, Science Service, Department of Agriculture, Ottawa.

The present investigation was undertaken to compare the occurrence of both the enterococcus group and the coliform group in commercially frozen samples of vegetables and cantaloupe.

METHODS

Over a period of one and one-half years, frozen foods were obtained from four plants in British Columbia and from one in Alberta, and tested for presence of both coliform and enterococcus organisms. The frozen foods were prepared for sampling by the method recommended in carrying out the plate count on these products, Report of the Committee on Standard Methods for the Microbiological Examination of Foods (1946). Fifty grams of sample were weighed into a sterile Waring Blendor and comminuted with 450 ml. of sterile water for two minutes. The resulting mixture was used in the presumptive coliform and enterococcus tests.

The standard presumptive coliform test was employed, using one-ml. quantities of the blended sample in duplicate tubes containing 10 ml. of brilliant green bile broth. Those tubes showing gas production within 48 hours of incubation at 37°C.(99°F.) were gram-stained and plated on eosin methylene blue agar.

The enterococcus presumptive test involved the use of the "S.F." medium developed by Hajna and Perry (1943), which contains sodium azide, an inhibitor of gram-negative bacteria, Lichstein (1941) and Snyder and Lichstein (1940). Duplicate tubes of "S.F." medium were inoculated with one-ml. quantities of the blended frozen foods and incubated at 45.5°C.(114°F.) in a constant temperature water bath for from 24 to 48 hours. Those tubes giving a positive test (acid production) were gram-stained and plated on "S.F." agar.

RESULTS

Seventy-nine "line" samples were obtained during the preparation of peas and beans for freezing and storage. These samples were taken after the blanch, during cooling, grading, and packing, and were held in storage no longer than one week. No evidence of either coliform or enterococci types could be found in samples taken immediately after blanching and so data are not presented for these samples. Of the other 62 samples, 82 per cent gave positive enterococcus tests, while 97 per cent showed coliform contamination.

In addition, 219 samples of frozen vegetables and cantaloupe were tested for coliform and enterococcus organisms, one to three months after packaging, and in this experiment the ratio of types was found to be reversed. Eighty-one per cent of the samples were enterococcus-positive, while only 75 per cent were coliform-positive.

A third experiment was carried out in order to test further the relative abilities of these two groups of organisms to survive in foods under cold storage conditions. Seventy samples of vegetables and cantaloupe containing enterococci and coliform organisms were retested after being held at —20°C.(—4°F.) for at least one year. It was found that 89 per cent still contained enterococci, while only 60 per cent contained coliform organisms.

Relative numbers of the two types of organisms in the three groups of frozen foods are shown in greater detail (Table 1).

Data presented (Table 1) show that coliform organisms continue to decrease in numbers during storage, while the enterococci remain fairly constant. As indicators of contamination, however, the coliform organisms appear to be superior to the enterococci in unstored or briefly stored foods, while the enterococci are a more reliable index in foods which have been held in cold storage. The "enterococcus+ coliform—" column (Table 1) will substantiate this conclusion.

Reliability of the two presumptive tests was checked by employing confirmatory tests on the coliform and enterococcus organisms isolated from eosin methylene blue agar and "S.F." agar, respectively. The indol, methyl red, Voges-Proskauer, and citrate tests were carried out on 508 coliform organisms obtained from 108 packages of frozen foods. Of those

TABLE 1

Occurrence of Coliform and Enterococcus Organisms in Commercial Samples of Frozen Vegetables and Cantaloupe Stored at —20°C.(—4°F.) for Varying Lengths of Time

Organisms	Percentage of samples showing coliform and enterococcus organisms		
	Samples stored 3 to 7 days	Samples stored 1 to 3 months	Samples stored 1 year
	pct.	*pct.*	*pct.*
Enterococcus+	82	81	89
Coliform+	97	75	60
Enterococcus+ coliform+	82	66	53
Enterococcus— coliform—	4	12	4
Enterococcus+ coliform—	0	13	36
Enterococcus— coliform+	15	7	7
Total number of samples	62	219	70

samples giving a positive presumptive test, only four per cent contained the fecal *E. coli*, while *A. aerogenes* was present in 68 per cent, and intermediate types were present in 81 per cent, of the samples. These percentages reveal the necessity for including the entire coliform group as the fecal indicator, rather than selecting *E. coli* to the exclusion of other members of the group.

Two hundred and sixty-two gram-positive cocci isolated from frozen foods on "S.F." agar were confirmed as belonging to the enterococcus group by employing the four tolerance tests of Sherman (1937): ability to grow in media containing 6.5 per cent sodium chloride; in media containing 0.1 per cent methylene blue; in broth at pH 9.5; and ability to reduce and clot litmus milk. In addition, 50 of these organisms were selected at random and further identified by the following tests: gelatin liquefaction; starch hydrolysis; aesculin splitting ability; final pH in glucose broth; ability to grow at 10°C.(50°F.); and fermentation studies with 10 sugars. Results, when compared with the classification of enterococci by Sherman (1937), showed that 86 per cent of the organisms were *Streptococcus fecalis*, while the remaining 14 per cent were *Streptococcus liquefaciens*. The confirmatory tests thus established the efficiency of the

"S.F." presumptive test for fecal streptococci. For use in routine work this test has proven to be as practical and easy to perform as the coliform presumptive test.

SUMMARY

Three hundred and seventy-six samples of commercially frozen vegetables and cantaloupe were examined for fecal contamination by use of presumptive coliform and enterococcus tests. The coliform test appears to be the more efficient of the two for detecting contamination in foods prior to freezing and storage, while the fecal streptococci are the superior indicators in frozen foods, as the coliform organisms seem less able to survive the storage temperatures. The presumptive enterococcus test, using Hajna and Perry's "S.F." medium, was found to be both reliable and practical in the routine examination of frozen foods.

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THIAMINE, RIBOFLAVIN, AND NIACIN CONTENT OF CHICKEN TISSUES, AS AFFECTED BY COOKING AND FROZEN STORAGE

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Relatively few reports of the effect of cooking and storage upon the nutritive value of poultry meat have appeared. Analyses of a few samples for thiamine by the rat-growth method, Booher and Hartzler (1939) and Micklesen, Waisman, and Elvehjem (1939), and by thiochrome procedure, Pyke (1939) and Leong (1939), have been reported, as have data on riboflavin determined by the microbiological method, Millares and Fellers (1949), and by fluorometry, Hodson (1940, 1941). Nicotinic acid occurrence as indicated by a chemical method, Dann and Handler (1942), McIntire, Waisman, Henderson, and Elvehjem (1941), and by both chemical and microbiological means, Waisman and Elvehjem (1941), has also been reported.

In few of these studies was there any systematic comparison of the vitamin concentration in the fresh and cooked or stored meat, or in the tissues of broilers as compared with fryers or fowl. Only a few studies of the effect of the composition of the diet of the birds upon that of the poultry meat have been reported. Hodson (1940) and Stamberg, Petersen, and Lampman (1947) examined the effect of variations in the level of dietary riboflavin; Denton, Kellogg, and Bird (1947) of nicotinic acid; and Pearson, Melass, and Sherwood (1946) of pantothenic acid. Millares and Fellers (1949) examined the vitamin and amino acid content of fresh, frozen, precooked frozen, canned and cured, smoked and canned light and dark meat of White Rock fowls. The meat was cooked by steaming for 40 minutes, then frozen and analyzed as soon as possible. No observations of the effect of frozen storage were included. Significant loss of thiamine was found to result from both cooking and processing, no loss of riboflavin in any case, and loss of niacin only in cured and smoked samples.

EXPERIMENTAL WORK

This study[1] was undertaken to determine the thiamine, riboflavin, and niacin content of all edible tissues in groups of birds produced on known practical feeds, and analyzed raw immediately after the birds were killed, after standardized cooking, and after having been held in frozen storage for from three to 12 months.

Method: The chickens were produced under known conditions of origin, feed, and growth. Three lots were examined: (1) single comb white Leghorns of both sexes,[2] 68 days old and weighing about one and one-half pounds, designated small broilers; (2) single comb white Leghorn cocker-

[1] The assistance of the Poultry and Egg National Board is gratefully acknowledged.

[2] Dr. H. J. Almquist, Poultry Husbandry Division, University of California, Berkeley, supplied the birds in Lot 1.

els, 60 days old, weighing two pounds, designated broilers; and (3) New Hampshire cockerels, 84 days old, weighing three pounds, and designated fryers.[3] A standard basal ration [4] was fed the small broilers and a somewhat similar ration [5] the broilers and fryers. Lots 1 and 2 were killed, bled, picked, and cooled on one day and eviscerated and shipped the next day. Lot 3 was killed, bled, picked, and eviscerated the same day, shipped the next day. They were wrapped, frozen, and stored at —23°C. (—9°F.) under standard conditions at the Western Regional Research Laboratory.

Broiling: One-half of each lot of birds were broiled in a well-regulated oven maintained at 160°C.(320°F.) and turned once, until the temperature of the interior of the thigh registered 85°C.(185°F.). The other half was used for analysis uncooked. All broiled portions were weighed before and after cooking. Water content of the various tissues was determined, raw and cooked, by heating in a vacuum oven at 70°C.(158°F.) until constant weight was attained. In most cases the ether extract was also obtained. Palatability of the cooked meat was judged by a group of five judges whose judgment had been found reliable. The hearts, livers, and gizzards were cooked in a double boiler in a constant amount of water, for 20 minutes. The cooking water was concentrated and added to the cooked samples for analysis.

For each set of determinations eight to 16 chickens were used. Some of the tissues of the smaller birds were combined for sampling, but at least three separate samples of each tissue were extracted and analyzed for every lot reported, except for the hearts, which were usually combined into one pool.

The Vitamin Analyses: Thiamine determinations were made by the thiochrome method with adsorption of the vitamin on an adsorbent according to the technique of Conner and Straub (1941). The weighed samples were ground or blended, extracted with 0.04 N H_2SO_4 at 100°C.(212°F.) for two hours, digested at 45°C.(113°F.) with Pangestin (0.5 gm. in 10 ml. sodium acetate buffer solution adjusted to pH 4.5) for two hours, made to volume, and filtered. Aliquots were oxidized.

Riboflavin determinations in most cases were obtained by both fluorometric and microbiological means. For the former the method of Peterson, Brady, and Shaw (1943) was used and for the latter that of Snell and Strong (1939). The extracts were made by boiling the samples with 0.04

[3] Dr. V. S. Asmundsen, Poultry Husbandry Division, University of California, Davis, supplied the birds of Lots 2 and 3.

[4] The percentage composition of the diet-fed Group I was as follows: Ground corn 25, wheat bran 13.25, ground barley 10, butyl fermentation residue 1 (0.25 mg. riboflavin per gm.), fish oil 0.25, soybean oil 2.5, bone meal 2, limestone 2, salt 1, soybean meal 15, sardine meal 3, alfalfa meal 15, wheat 10. To 75 parts of this ration, 25 parts of a mixture of alfalfa meal, 11 to 15, and wheat one to 14 parts were added. In eight weeks the chicks fed this ration weighed 555 gm. and had gained 0.3 gm. per gm. feed eaten. This ration contained in mg. per cent, riboflavin 0.217, thiamine 0.292, and niacin 2.54.

[5] Groups 2 and 3 were fed a somewhat similar ration made up as follows: dried milk, alfalfa meal, soybean meal, each 5, sardine 12.5, wheat bran 10, wheat 30, barley 12.5, yellow corn 15, bone meal 1, limestone 2, salt 0.5, sardilene 0.125, dried molasses 1.5, $MnSO_4 \cdot 4H_2O$ 0.025. This ration contained in mg. per cent, riboflavin 0.217, thiamine 0.332, and niacin 2.22.

N H_2SO_4 for 30 minutes, grinding or blending, then autoclaving for 30 minutes at 15 pounds' pressure. The pH was then adjusted to 4.5 with NaOH, the volume made to 100 ml., and the solution filtered. The pH of the filtrate was brought to seven and this extract used for the assays. In the case of the liver samples further treatment with Clarase in sodium acetate buffer at pH 4.5 for two hours at 45°C. was found to be necessary. The skin and breast samples were extracted with 0.5 N H_2SO_4, instead of 0.04 N H_2SO_4.

Niacin determinations were made by the microbiological method of Snell and Wright (1941). Portions of the same extracts used for the microbiological assay for riboflavin were used for the niacin analyses.

Changes due to cooking are shown (Tables 1 and 2) and results of the vitamin analyses are presented (Tables 3, 4, 5, 6, and 7).

DISCUSSION

Cooking Losses: Loss of weight in cooking, which varied from 18.5 to 29.2 per cent, was not significantly affected by length of storage or by size of the chickens (Table 1). This differed from the findings with tur-

TABLE 1

Total Weights of Raw and Broiled Chickens

Lot	Storage	No. of birds	Average weight		Loss in cooking
			Raw	Broiled	
	mo.		*gm.*	*gm.*	*pct.*
1	0	12	420	315	25.0
	4	8	410	334	18.5
	8	16	445	315	29.2
2	0	12	520	400	23.0
	4	12	526	382	27.3
	8	8	510	372	27.0
	12	8	523	402	23.1
3	0	12	1020	825	19.1
	4	12	1052	795	24.4
	8	8	1034	821	20.6
	12	8	1061	815	23.2

keys, Cook, Morgan, and Smith (1949), in which weight loss in cooking was increased after storage and decreased in the larger birds. Changes in water and fat content of the chicken tissues, due to cooking, followed the usual pattern of loss of water in all samples, and loss of fat in fatty tissues, or small apparent gains in fat due to moisture changes or seepage of fat from the fatter portions (Table 2).

Loss of thiamine caused by cooking was significant and fairly consistent (Table 3), usually amounting to from 20 to 40 per cent. Riboflavin and niacin were better retained in the cooked meat than was thiamine (Tables 4 and 5). With only a few exceptions losses of both riboflavin and niacin ranged from 10 to 20 per cent of the content of the raw meat. Variation in these cooking losses was inevitable, since the size of the broilers used in the various lots, and therefore surface heat exposure, varied considerably. Concentration of thiamine in the breast, gizzard, and skin was so

TABLE 2

Typical Analyses of Raw and Cooked Chicken Tissues

Lot	Tissue	Water		Fat	
		Raw	Cooked	Raw	Cooked
		pct.	*pct.*	*pct.*	*pct.*
1	Leg	74.6	67.3	3.6	4.2
	Breast	74.2	69.1	0.5	1.5
	Skin	53.8	46.2	28.1	24.7
	Liver	74.1	68.5	2.7	3.8
	Gizzard	75.1	68.2	6.9	3.6
	Heart	74.4	69.2	7.0	6.7
2	Leg	76.3	72.8	3.3	3.6
	Breast	74.1	70.0	0.6	1.0
	Skin	55.1	49.8	24.0	22.6
	Liver	75.0	71.2	1.6	2.3
	Gizzard	69.6	64.4	2.9	2.6
	Heart	72.7	63.3	6.7	5.7
3	Leg	77.4	70.6	1.0	2.6
	Breast	75.7	70.1	0.3	0.5
	Skin	60.2	52.6	19.5	19.6
	Liver	73.0	69.9	1.9	2.9
	Gizzard	76.9	72.1	2.1	2.3
	Heart	78.3	76.4	3.8	3.9

low as to render doubtful the validity of retention differences. Average retentions of the vitamins during cooking are shown (Table 6).

Distribution of the Vitamins: The breast muscles of the chicken represent tissue less likely to be used, that is, less active in energy transformations than the leg muscles. As had been noted previously by others, the breast muscle contained less riboflavin and thiamine, and more niacin, than the leg muscle. The gizzard, which is an active muscular organ, had little thiamine and niacin, but was well endowed with riboflavin, ranking next to liver and heart in this regard.

The skin of the white Leghorns also contained little thiamine, but that of the New Hampshire chickens contained as much as the leg and heart. Loss was rapid in storage. The riboflavin of the skin was of the order of that in the leg, and the niacin similar to that of the breast. Losses in storage and cooking were irregular and comparable with those of the leg and breast.

The heart of these chickens contained about the same amount of thiamine and niacin as the leg muscles, but three or four times as much riboflavin. In a parallel study of turkey tissues, Cook, Morgan, and Smith (1949), the heart was found to be not only rich in riboflavin but also to contain more thiamine than any of the other tissues, not excluding the liver. The lower thiamine content of the chicken hearts may be due to the fact that these birds were considerable younger than the turkeys, or there may be a species difference. Rice, Strandine, Squires, and Lydden (1946) reported values for thiamine in the hearts of three mature chickens as 2.2, 2.1, and 0.9 μg. per gm., values similar to those found in our turkey experiment.

Thiamine concentration of the breast muscle of these chickens ranged from 0.04 to 0.06 mg. per cent, and the leg muscle from 0.06 to 0.07 mg.

TABLE 3

Thiamine in Chicken Tissues[1,2]

Tissue	Storage	Lot 1			Lot 2			Lot 3		
		Raw	Cooked	Retention	Raw	Cooked	Retention	Raw	Cooked	Retention
	mo.			*pct.*			*pct.*			*pct.*
Leg muscles	0	0.08	.....	.....	0.07	0.06	85	0.05	0.04	80
	4	0.05	0.03	60	0.06	0.03	50	0.04	0.03	75
	8	0.05	0.04	60	0.06	0.04	67	0.06	0.04	67
	12	.....	.....	.....	0.06	0.03	50	0.06	0.03	50
Breast muscles	0	0.06	.....	.....	0.05	0.03	60	0.04	0.03	75
	4	0.02	0.02	100	0.05	0.03	60	0.03	0.03	100
	8	0.02	0.02	100	0.04	0.04	100	0.04	0.03	75
	12	.....	.....	.....	0.02	0.01	50	0.03	0.03	100
Liver	0	0.24	.....	.....	0.31	0.13	42	0.23	0.14	61
	4	0.13	0.09	69	0.24	0.12	50	0.22	0.15	69
	8	0.14	0.09	64	0.16	0.10	62	0.23	0.17	74
	12	.....	.....	.....	0.13	0.12	92	0.21	0.15	71
Heart	0	0.12	.....	.....	0.05	0.04	80	0.05	0.05	100
	4	0.06	0.04	67	0.06	0.04	67	0.06	0.04	67
	8	0.05	0.04	80	0.04	0.04	100	0.07	0.06	86
	12	.....	.....	.....	0.02	0.02	100	0.05	0.04	80
Gizzard	0	0.01	.....	.....	0.02	0.01	.....	0.02	0.02	.....
	4	0.01	0.00	.....	0.02	0.01	.....	0.02	0.02	.....
	8	0.01	0.01	.....	0.02	0.01	.....	0.02	0.02	.....
	12	.....	.....	.....	0.02	0.01	.....	0.02	0.02	.....
Skin	0	.....	.....	.....	0.02	0.01	.....	0.08	0.03	37
	4	0.00	0.00	.....	0.02	0.01	.....	0.04	0.02	50
	8	0.00	0.00	.....	0.02	0.01	.....	0.03	0.02	67
	12	.....	.....	.....	0.01	0.00	.....	0.02	0.02	100

[1] In mg. per 100 gm. fresh weight or equivalent. [2] Each figure represents the average of determinations in at least three pools of two to four chickens each.

per cent. This agrees well with the 0.051 mg. per cent reported by Pyke (1939), who also used the thiochrome procedure. As is usually the case, the apparent thiamine value of these tissues was considerably larger as determined by rat-growth method. By the latter method, values of 0.078 and 0.111 mg. per cent were found by Booher and Hartzler (1939) for breast and leg, respectively; 0.07 and 0.15 for breast and 0.18 and 0.27 for leg by Mickelsen *et al.* (1939). The same contrast was seen in the turkey study, Cook, Morgan, and Smith (1949), in which fresh samples of leg and breast meat were found by rat-growth to yield about one-third more thiamine than by the chemical procedure, but samples stored three months appeared to have the same thiamine content when analyzed by the two methods.

Tissues of the birds used by Millares and Fellers (1949) were found to contain considerably more thiamine than any of the lots used in this experiment. Their birds were White Rock fowls, probably much older and larger than any of our lots. Riboflavin and niacin values were of the same order as found in our study.

Thiamine content of the active muscles, leg and heart, exceeded that of the inactive breast muscles, the skin, and the gizzard, but the differences were not large, the ratio of thiamine in the two groups of tissues did not exceed two or three to one. On the other hand, the active muscles of heart, gizzard, and leg contained three to 10 times as much riboflavin as the inactive breast muscle and skin. Relationship of activity to riboflavin content appears clear (Tables 3, 4, and 5).

Niacin present in all the tissues except liver and breast was of the same order, four to seven mg. per cent of fresh tissue. Whether both liver and breast niacin represent storage, or a specific function of the niacin-containing co-enzymes in the metabolism of these tissues, remains to be determined.

EFFECT OF DIET ON VITAMIN CONTENT OF TISSUES

There was no significant difference in the thiamine content of the two diets used and little difference among the tissues of the three lots of birds (Table 3).

The riboflavin content of the feeds used in this experiment, 2.17 μg. per gm., was apparently adequate since the levels of this vitamin found in the

TABLE 4

Riboflavin in Chicken Tissues[1,2]

Tissue	Storage	Lot 1			Lot 2			Lot 3		
		Raw	Cooked	Retention	Raw	Cooked	Retention	Raw	Cooked	Retention
	mo.			*pct.*			*pct.*			*pct.*
Leg muscles	0	0.27	0.21	78	0.22	0.18	82	0.18	0.09	50
	4	0.26	0.26	100	0.16	0.13	81	0.19	0.08	42
	8	0.26	0.26	100	0.15	0.15	100	0.15	0.15	100
	12	.....	.....	.....	0.09	0.10	111	0.11	0.12	109
Breast muscles	0	0.07	0.10	104	0.09	0.07	78	0.08	0.06	75
	4	0.06	0.07	116	0.11	0.06	54	0.05	0.08	160
	8	0.07	0.09	128	0.09	0.04	44	0.04	0.04	100
	12	.....	.....	.....	0.11	0.07	63	0.06	0.04	67
Liver	0	3.20	3.00	93	3.10	1.60	51	2.83	2.41	85
	4	3.17	2.43	77	2.77	1.64	68	2.80	2.20	78
	8	3.00	2.80	93	2.93	1.50	51	2.30	2.44	106
	12	.....	.....	.....	2.23	1.61	72	1.47	1.51	103
Heart	0	1.10	1.10	100	1.00	0.80	80	0.50	0.43	86
	4	0.80	0.70	87	0.70	0.65	93	0.70	0.38	54
	8	0.90	0.74	82	0.70	0.44	63	0.60	0.60	100
	12	.....	.....	.....	0.50	0.38	76	0.46	0.45	98
Gizzard	0	0.01	0.00	.....	0.26	0.12	46	0.19	0.17	89
	4	0.01	0.00	.....	0.17	0.14	82	0.16	0.19	110
	8	0.01	0.01	.....	0.22	0.11	50	0.18	0.14	77
	12	.....	.....	.....	0.15	0.13	87	0.19	0.16	84
Skin	0	.....	.....	.....	0.11	0.11	100	0.16	0.16	100
	4	0.17	0.17	100	0.12	0.08	67	0.10	0.15	150
	8	0.19	0.20	105	0.11	0.07	63	0.08	0.09	112
	12	.....	.....	.....	0.10	0.09	90	0.07	0.05	71

[1] In mg. per 100 gm. fresh weight or equivalent. [2] Each figure represents the average of determinations in at least three pools of two to four chickens each.

TABLE 5

Niacin in Chicken Tissues[1,2]

Tissue	Storage	Lot 1			Lot 2			Lot 3		
		Raw	Cooked	Retention	Raw	Cooked	Retention	Raw	Cooked	Retention
	mo.			*pct.*			*pct.*			*pct.*
Leg muscles	0	5.4	3.8	70	4.6	4.1	89	5.2	4.1	80
	4	5.0	3.9	78	4.9	3.6	73	4.9	3.6	73
	8	4.9	4.5	92	4.3	3.3	77	4.2	3.6	85
	12	.....	.....	.....	3.7	2.8	75	3.0	2.4	80
Breast muscles	0	9.2	7.6	82	9.6	6.9	72	10.2	9.3	91
	4	8.7	7.8	90	9.5	7.0	73	9.8	8.0	82
	8	8.4	8.8	105	8.5	7.7	90	9.4	8.6	91
	12	.....	.....	.....	9.6	3.8	40	9.1	8.1	89
Liver	0	12.9	10.4	80	8.4	4.6	54	9.0	7.3	81
	4	11.5	9.6	83	8.5	5.4	63	8.8	6.6	75
	8	11.5	11.5	100	8.1	5.4	67	8.5	8.0	94
	12	.....	.....	.....	8.1	6.2	77	7.7	8.0	104
Heart	0	4.5	4.7	104	5.0	3.8	76	6.5	4.0	61
	4	3.9	3.4	87	5.2	3.9	75	4.6	3.4	74
	8	4.0	3.7	92	4.8	3.8	80	3.9	4.0	100
	12	.....	.....	.....	3.4	2.9	74	3.2	3.5	109
Gizzard	0	5.0	4.3	86	4.1	.....	.....	5.6	4.8	85
	4	4.7	3.9	83	3.1	3.3	106	5.2	4.7	90
	8	4.6	3.7	82	3.5	3.0	85	5.2	4.9	94
	12	.....	.....	.....	3.4	2.7	80	4.1	3.7	90
Skin	0	.....	.....	.....	9.6	3.8	40	7.6	4.9	64
	4	6.8	6.7	98	8.2	5.6	68	6.9	4.8	70
	8	6.1	7.5	123	9.6	3.8	40	6.6	5.8	88
	12	.....	.....	.....	8.9	3.8	42	5.6	4.2	75

[1] In mg. per 100 gm. fresh weight or equivalent. [2] Each figure represents the average of determinations in at least three pools of two to four chickens each.

livers, breast, and leg muscle were similar to those reported by Stamberg *et al.* (1947) for feeds which contained from two to five times as much riboflavin. None of the tissues, except the breast, however, exhibited the high riboflavin content reported by Hodson (1940) in chickens fed a diet containing 6.9 μg. per gm. (Table 4).

Niacin of the two diets used in this experiment differed only slightly, 22.2 μg. per gm. in the diet fed Group 1, and 25.4 in that fed Groups 2 and 3. This level was apparently below the optimum, since all the samples yielded niacin values in the lowest ranges reported by Denton *et al.* (1947) for birds produced on feeds containing 28 μg. per gm., the lowest level of niacin which they used. The decrease in niacin of liver, leg, and breast meat with age as reported by these investigators was not discernible in our experiment, perhaps because of the somewhat lower niacin content of the diet given Group 1 (Table 5).

Losses in Storage: Thiamine loss in storage was small except in the tissues of Lot 1, the smallest birds used, although not the youngest. Riboflavin losses in breast, leg, and liver were negligible during eight months' storage, except in breast of Lot 3 and leg of Lot 2 (Table 7).

TABLE 6

Average Retention of Vitamins[1,2] by Cooked Chicken Tissues

Lot	Tissue	Thiamine			Riboflavin			Niacin		
		Raw	Cooked	Pct.	Raw	Cooked	Pct.	Raw	Cooked	Pct.
1	Breast	0.03	0.02	67	0.07	0.08	114	8.8	8.0	91
2	Breast	0.05	0.03	60	0.09	0.06	67	9.3	7.3	78
3	Breast	0.04	0.03	75	0.06	0.06	100	9.6	8.5	89
1	Leg	0.05	0.04	80	0.26	0.24	92	5.1	4.1	80
2	Leg	0.06	0.04	67	0.16	0.14	88	4.3	3.5	81
3	Leg	0.05	0.04	80	0.16	0.11	69	4.3	3.4	80
1	Liver	0.17	0.09	53	3.12	2.74	88	12.0	10.5	87
2	Liver	0.21	0.12	59	2.75	1.59	58	8.3	5.4	65
3	Liver	0.22	0.15	68	2.35	2.14	91	8.5	7.5	88
1	Heart	0.06	0.04	67	0.93	0.85	91	4.1	3.9	95
2	Heart	0.04	0.03	75	0.72	0.57	80	4.6	3.6	79
3	Heart	0.06	0.05	83	0.56	0.46	82	4.5	3.7	82
1	Gizzard	0.01	0.00	....	0.23	0.21	91	4.8	4.0	83
2	Gizzard	0.02	0.01	....	0.20	0.12	60	3.5	3.0	86
3	Gizzard	0.02	0.02	....	0.18	0.16	89	5.0	4.5	90
1	Skin	0.00	0.00	....	0.18	0.19	105	6.5	7.0	107
2	Skin	0.02	0.01	50	0.11	0.09	82	9.1	4.4	48
3	Skin	0.04	0.02	50	0.11	0.11	100	6.7	4.9	73

[1] All vitamins are expressed in mg. per 100 mg. fresh tissue or equivalent. [2] Forty birds were used in each of Lots 2 and 3; 36 in Lot 1.

TABLE 7

Retention of Vitamins by Chicken Tissues in Frozen Storage

No. of Birds	Storage	Tissue	Thiamine			Riboflavin			Niacin		
			Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3
	mo.		*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*
32	4	Breast	35	87	89	86	128	63	95	100	96
32	8	Muscle	35	96	109	100	157	50	92	100	92
16	12		....	83	91	....	128	75	....	85	90
32	4	Leg	66	87	85	100	73	105	97	106	95
32	8	Muscle	66	83	117	100	69	83	89	94	81
16	12		....	83	117	....	40	61	....	81	55
32	4	Liver	55	79	95	99	90	100	89	100	98
32	8		59	50	100	94	94	82	89	97	95
16	12		....	40	90	....	72	53	....	97	85
32	4	Heart	50	117	120	73	70	140	87	104	71
32	8		42	86	140	82	70	120	89	96	60
8	12		....	38	....	....	50	92	....	68	50
32	4	Gizzard	59	100	100	77	66	84	94	76	93
32	8		50	82	100	92	85	95	92	85	93
16	12		....	68	100	....	58	100	....	83	74
24	4	Skin	....	100	50	100	100	63	90	85	91
16	8		....	90	40	....	100	50	....	100	87
16	12		....	65	22	....	90	25	....	93	74

The niacin content of breast and leg muscles was not significantly changed during storage up to eight months. After 12 months definite losses occurred, particularly in the leg muscle.

SUMMARY

Thiamine, riboflavin, and niacin were determined by chemical and microbiological methods in the leg and breast muscles, liver, heart, gizzard, and skin of 116 young chickens, 60 to 84 days old. All tissues were examined, both uncooked and cooked, shortly after slaughter and after four, eight, and 12 months' frozen storage.

In the fresh, raw samples the active muscles of leg, heart, and gizzard contained 0.18 to 1.10 mg. per cent riboflavin, three to 10 times as much as did the inactive breast muscle and skin. The liver contained 2.8 to 3.2 mg. per cent riboflavin.

The leg muscle and heart had 0.05 to 0.12 mg. per cent thiamine, which was two to three times as much as found in the breast and skin. The gizzard was found to be unusually poor in thiamine. The liver contained 0.23 to 0.31 mg. per cent thiamine.

Niacin was fairly evenly distributed in all tissues, four to seven mg. per cent, except in breast, skin, and liver, which were about equal in value, eight to 12 mg. per cent.

Loss of weight in cooking varied from 18.5 to 29.2 per cent, with loss in water and slight gains or losses in fat of the various tissues. Twenty to 40 per cent of the thiamine disappeared in the cooked meat and usually 10 to 20 per cent of the riboflavin and niacin.

The riboflavin of the feed used for the production of these chickens, 2.17 μg. per gm., was adequate to insure maximum riboflavin content of the tissues. The thiamine of the feed, 3.32 μg. per gm., apparently was adequate also, but the levels of niacin, 22.2 and 25.4 μg. per gm., must have been below the optimum, since all the tissues were in the lower range of niacin content.

Loss of niacin in frozen storage up to eight months was negligible in nearly all tissues, but in some cases losses of 25 to 50 per cent appeared after 12 months' storage. Riboflavin was well retained in most cases up to eight months, with the exception of breast meat and skin in one lot and leg meat in another. Thiamine in all tissues of Lot 1 appeared to be labile, although riboflavin and niacin of this lot were stable. There was little loss of thiamine in storage in the other two lots of tissues.

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THIAMINE, RIBOFLAVIN, AND NIACIN CONTENT OF TURKEY TISSUES AS AFFECTED BY STORAGE AND COOKING[1]

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There have now appeared several reports of the vitamin content of chicken tissues, but few as to distribution of vitamins in turkey meat. The present study was undertaken in order to determine whether thiamine, riboflavin, and niacin are distributed similarly in turkey and chicken tissues; whether these vitamins are unstable or extractable during the usual turkey roasting process; and whether frozen storage for from three to nine months produces changes in vitamin concentration.

Incidental observations were made on presence or absence of flavors in the meat of birds fed varying amounts of fish meal. A parallel study of the effect of feeding varying amounts of tocopherols, on development of rancidity in the tissues of the stored birds, was made in two of the three experiments reported. A similar study of vitamin retention during cooking and storage of chicken tissues, Morgan, Kidder, Hunner, Sharokh, and Chesbro (1949), had previously been made in this laboratory, using similar procedures.

EXPERIMENTAL PROCEDURE

Three lots of turkeys were examined. Lot 1, consisting of 32 turkeys of the Bronze strain, of from 27 to 30 weeks of age, was divided into two groups—each containing eight male and eight female birds. Tissues of one of these groups were studied, raw and cooked, when the birds were freshly killed, and those of the other group after three months' frozen storage at −23°C.(−9°F.). Lots 2 and 3, all females of the same strain, 34 and 36 weeks old, were fed somewhat similarly. Lot 2 consisted of 16 birds, divided into four groups of four each, examined raw and cooked when freshly killed and after three, six, and nine months' frozen storage. Lot 3 also contained 16 birds, five of which were examined when freshly killed, five after three months, and six after five months' frozen storage.

FEEDING AND PREPARATION OF TURKEYS

Lot 1 was fed a basal mash as 68 per cent of the diet and a protein supplement as 32 per cent. The 68 parts of basal mixture contained: alfalfa meal 7.5, dried whey 7.5, wheat 10.0, wheat bran 15.0, corn 7.0, barley 10.0, fish meal 7.5, limestone 2.0, salt 1.0, fish oil 400D 0.5, and manganese sulfate 0.025. The supplement was made up in four different ways, designated Diets 1, 3, 6, and 9, respectively. Supplement 1 con-

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tained: cottonseed meal 30, bone meal 2; Supplement 3, soybean meal 30, bone meal 2; Supplement 6, cottonseed meal and soybean meal each 10, fish meal and barley each 5.5, bone meal 1; Supplement 9 contained cottonseed meal and soybean meal each 5, fish meal and barley each 11. These different diets were fed to determine whether use of fish meal in varying amounts might affect the flavor and acceptability of the meat.

Lots 2 and 3 were fed a similar mixture. The basal mash and grain supplements were fed in equal parts. Composition of the resulting mixture was alfalfa meal 7.5, wheat 32.5, wheat bran 10.0, corn 7.0, barley 25.0, fish meal 5.0, soybean meal 10.0, limestone and bone meal 2.0, salt 0.5, fish oil 400D 0.5, and manganese sulfate 0.025. This is quite close in composition to both Diets 6 and 9 used for Lot 1.

All turkeys were bled, soft-scalded, eviscerated, and placed in a cooler at 20°C.(68°F.) overnight, then wrapped in locker wrap paper, kept at −34°C.(−29°F.) for three days, and stored thereafter at −23°C.(−9°F.) at the U. S. Department of Agriculture Western Regional Research Laboratory in Albany, California.

COOKING METHOD

Analysis of both fresh-killed and cold-storage birds was made on raw and cooked tissues of each bird. Each turkey was cut in half. Raw tissues were taken from one side and the other side was cooked after canvas had been clamped to the exposed areas. No salt, seasonings, or stuffings were used in any case. Each half-turkey was placed on a rack in a shallow, uncovered pan, canvas side down, in a separate oven, and the pan turned at intervals of from 20 to 30 minutes to insure an even degree of cooking on all sides. Time and temperature of roasting were adapted to weight of the bird according to directions given by Alexander (1941). Most of the half-turkeys required two to three hours' cooking at 168°C.(334°F.).

PALATABILITY TESTS

The cooked turkey meat was judged for palatability, while hot, by from six to eight judges. White meat samples were taken from the breast, dark meat from the thigh, and skin and fat samples from the back. Judges scored texture, flavor, and aroma of these tissues as desirable, moderately desirable, slightly desirable, slightly undesirable, and undesirable. In addition, they were asked to describe any off-flavors and aromas detected. In Lots 2 and 3 they attempted particularly to detect the presence of rancidity.

SAMPLING

The muscle of leg and breast was dissected away from bone, fat, and skin and put through a fine grinder twice. The skin of the back was separated from the fat and cut with scissors into small pieces; the liver was similarly finely divided; heart and gizzard were shredded with a razor blade. Moisture, fat, and vitamin determinations were made on all raw tissues, but on only the leg and breast muscle and liver of the cooked samples.

METHODS OF VITAMIN ANALYSIS

Thiamine, riboflavin, and niacin were determined in the same extracts of samples obtained on Lots 2 and 3. Samples were blended with 0.1N

H_2SO_4, then steamed for one hour, pH adjusted to 4.5, and the mixture incubated overnight at 37°C.(99°F.) with 0.25 gm. Pangestin in 10-ml. acetate buffer. Extracts were made to volume and filtered. The leg, liver, heart, and skin filtrates were washed with three portions of chloroform and the chloroform removed by centrifuging. In several cases the skin samples were judged to be too fat for vitamin analysis.

Thiamine was determined in these filtrates by the thiochrome method of Conner and Straub (1941); riboflavin was measured microbiologically by the method of Snell and Strong (1939); and niacin by the method of Snell and Wright (1941).

Tissues of Lot 1 were examined for thiamine by the rat-growth method, Jentsch and Morgan (1949), and for riboflavin chiefly by the microbiological procedure used for Lots 2 and 3, but also in some cases by chick-growth assay and by fluorometry, Peterson, Brady, and Shaw (1943). Niacin was determined by the same method used for Lots 2 and 3.

TABLE 1

Weight Losses in Cooking Half-Turkeys

Lot	Number birds	Storage	Wt. half-turkey		Wt. of drippings	Total cooking loss	Loss by evaporation	Sex
			Raw	Cooked				
		mo.	*gm.*	*gm.*	*gm.*	*pct.*	*pct.*	
1	4	0	5427	4140	425	23.7	15.8	Male
	8	....	2866	1951	345	31.9	19.9	Female
	8	3	4971	3458	338	30.4	23.8	Male
	8	....	2806	1742	202	38.0	30.8	Female
2	3	0	2240	1720	.....	.....	23.2	Female
	4	3	2205	1560	.....	.....	29.3	Female
	4	6	2120	1176	138	44.5	38.0	Female
	4	9	1881[1]	1231	146	34.5	26.9	Female
3	5	0	2442	1625	.....	.....	33.5	Female
	5	3	1807[1]	1205	237	33.3	20.2	Female
	6	5	1967[1]	1300	209	33.9	23.3	Female

[1] The leg and thigh had been removed from these half-turkeys for another analysis before cooking.

The process used for the preparation of extracts of tissues of Lot 1, for use in the microbiological analysis for riboflavin, differed somewhat from that described above for Lots 2 and 3. After the samples were blended and steamed with 0.1N H_2SO_4 they were digested at from 45 to 50°C.(113 to 122°F.) with one per cent pancreatin solution at pH 7 in phosphate buffer for two hours, filtered, and made to volume. Incubation with pepsin or papain and takadiastase at pH 4.5 for two or 22 hours had been found to yield less consistent values than the procedure finally adopted. Pangestin was not used in this series.

RESULTS AND DISCUSSION

Weight Loss During Cooking: Loss in weight which occurred during cooking is shown (Table 1). The apparently smaller loss in male than female birds shown in Lot 1 may be due only to the greater size of the former. Accurate measurement of drippings was difficult in case of the

larger birds, due to spattering. In the group of birds stored nine months in Lot 2, and those stored three and five months in Lot 3, the leg and thigh were removed before cooking because they were needed for a subsequent set of analyses. It may be noted that this procedure decreased loss by evaporation and increased the loss as drippings, as compared with changes shown by the intact half-turkeys. This was doubtless due to the decrease in total surface resulting from removal of leg and thigh. In comparable lots the total loss and loss by evaporation were always greater in the stored birds than in those freshly killed. Cooking losses increased progressively with length of frozen storage period.

TABLE 2

Thiamine in Turkey Tissues[1]

Tissue	Storage	Lot 1	Lot 2	Lot 3
	mo.			
Leg muscles	0	0.14[2]	0.09	0.11
	3	0.08[2]	0.08	0.10
	5	.....	.....	0.09
	6	.....	0.10	.....
	9	.....	0.10	.....
Breast muscles	0	0.11[2]	0.06	0.05
	3	0.07[2]	0.07	0.07
	5	.....	.....	0.07
	6	.....	0.07	.....
	9	.....	0.08	.....
Liver	0	0.23[2]	0.10	.....
	3	.....	.....	0.12
	5	.....	.....	0.21
	6	.....	.....	.....
Heart	0	.....	0.22	0.24
	3	.....	0.23	.....
	5	.....	.....	0.19
	6	.....	0.22	.....
	9	.....	0.21	.....
Gizzard	0	.....	0.04	0.06
	3	.....	0.05	0.06
	5	.....	.....	0.05
	6	.....	0.04	.....
	9	.....	0.04	.....
Skin	0	.....	0.03	.....
	3	.....	0.03	.....

[1] In mg. per 100 gm. fresh weight or equivalent. [2] These values were determined by the rat-growth method, all others by thiochrome measurement.

Palatability: Palatability tests of Lot 1 demonstrated there was an off-flavored fishy quality in both fresh and stored turkeys from groups which received Supplements 6 and 9. This off-flavored quality was attributed to the 13 and 18.5 per cent levels of sardine meals fed in the total rations. Asmundson, Jukes, Fyler, and Maxwell (1938) who fed rations containing 25 per cent sardine or tuna fish meal, found that, if the birds were not starved prior to killing, and were held in a warm room for from 12 to 24 hours before evisceration, flavor was generally adversely affected.

It is unlikely that development of the undesirable flavor of the tissues in the present experiment could be attributed to the preparation of the turkeys, since they were starved overnight, immediately drawn, and placed in a cooler. Turkeys on Supplements 1 and 3, which received either cottonseed or soybean meal and only 7.5 per cent of sardine meal, were judged to have excellent flavor, aroma, and texture. Freezing storage for three months produced no significant change in flavor of the birds of Lot 1.

TABLE 3

Riboflavin in Turkey Tissues[1]

Tissue	Storage	Lot 1	Lot 2	Lot 3
	mo.			
Leg	0	0.29	0.18	0.12
	3	0.28	0.14	0.15
	5	.....	.....	0.17
	6	.....	0.13	.....
	9	.....	0.13	.....
Breast	0	0.16	0.11	0.11
	3	0.16	0.10	0.10
	5	.....	.....	0.09
	6	.....	0.11	.....
	9	.....	0.09	.....
Liver	0	3.20	2.28	.....
	3	.....	.....	1.11
	5	.....	.....	2.05
	6	.....	0.76	.....
Heart	0	.....	0.63	1.08
	3	.....	1.07	.....
	5	.....	.....	0.88
	6	.....	0.92	.....
	9	.....	0.90	.....
Gizzard	0	.....	0.11	0.17
	3	.....	0.15	0.19
	5	.....	.....	0.18
	6	.....	0.14	.....
	9	.....	0.17	.....
Skin	0	.....	0.05	.....
	3	.....	0.09	.....

[1] In mg. per 100 gm. fresh weight or equivalent.

In Lots 2 and 3 varying amounts of tocopherols[2] had been fed the turkeys from two to 35 days before slaughter, to observe any antioxidant effects which might be thus induced in the tissues during storage. Rancidity of thigh meat and fat was noted in several of the birds on the low tocopherol dosages, particularly after from five to nine months' storage. Only two of six birds on tocopherol-free or tocopherol-low diet were judged to have rancid thigh meat after three months' storage, but seven out of 12 after five to nine months' storage.

Distribution of Vitamins Among Tissues: As was found also in the chicken study, Morgan *et al.* (1949), thiamine and riboflavin were more

[2] This study will be presented in full in a separate communication.

concentrated in the leg muscles, and niacin in the breast muscles, of the turkeys (Tables 2, 3, and 4).

The heart had the most thiamine of any tissue, 0.22 mg. per cent; the liver the next highest content, 0.14; then the leg muscle, 0.09; breast muscle 0.07; and last the gizzard and skin, 0.04 and 0.03, respectively. In the chicken study, smaller concentrations were found in all tissues, possibly due to the fact that those birds were much younger than the turkeys (Table 2).

TABLE 4

Niacin in Turkey Tissues[1]

Tissue	Storage	Lot 1	Lot 2	Lot 3
	mo.			
Leg	0	4.8	3.6	5.6
	3	5.5	4.1	5.1
	5	.....	.....	5.4
	6	.....	4.3	.....
	9	.....	4.9	.....
Breast	0	11.0	10.5	12.5
	3	10.4	11.6	12.1
	5	.....	.....	11.5
	6	.....	10.6	.....
	9	.....	11.3	.....
Liver	0	15.1	11.2	.....
	3	15.3	.....	9.9
	5	.....	.....	10.9
	6	.....	11.0	.....
Heart	0	.....	4.5	5.4
	3	.....	6.1	.....
	5	.....	.....	7.1
	6	.....	7.2	.....
	9	.....	7.0	.....
Gizzard	0	.....	4.4	5.7
	3	.....	5.6	5.6
	5	.....	.....	4.5
	6	.....	5.2	.....
	9	.....	6.1	.....
Skin	0	.....	4.6	.....
	3	.....	3.0	.....

[1] In mg. per 100 gm. fresh weight or equivalent.

The turkey livers contained variable amounts of riboflavin, about one to three mg. per cent, somewhat less than in chicken livers. The hearts had 0.91 mg. per cent, leg muscles and gizzard each 0.15, breast muscles 0.10, and skin, 0.07 (Table 3). In the chickens, leg, skin, and gizzard contained more, and breast muscles less, riboflavin than in the turkeys.

Niacin was most concentrated in the breast muscles and liver of the turkeys and in about the same amount, slightly more than was seen in the chicken liver and breast. The heart, gizzard, leg muscles, and skin had decreasing contents of niacin; larger in each case, however, than in the corresponding chicken tissues, except for skin (Table 4).

TABLE 5

Changes in Composition of Turkey Tissues Due to Cooking

Lot	Number birds	Tissue	Water		Fat		Thiamine		Retention	Riboflavin		Retention	Niacin		Retention
			Raw	Cooked	Raw	Cooked	Raw	Cooked [2]		Raw	Cooked		Raw	Cooked	
			pct.	*pct.*	*pct.*	*pct.*	*mg. pct.*	*mg. pct.*	*pct.*	*mg. pct.*	*mg. pct.*	*pct.*	*mg. pct.*	*mg. pct.*	*pct.*
1	32	Breast muscle	73.0	63.6	.....	.....	0.09[1]	0.06[1]	67	0.16	0.13	81	10.7	7.4	70
2	8	Breast muscle	73.5	63.9	0.6	2.5	0.07	0.04	57	0.11	0.08	72	11.0	8.5	77
3	5	Breast muscle	72.7	64.0	1.0	1.9	0.06	0.04	62	0.11	0.09	82	12.5	9.0	72
1	32	Leg muscle	74.8	61.1	.....	.....	0.11[1]	0.05[1]	45	0.28	0.22	78	5.1	3.5	68
2	8	Leg muscle	74.5	57.8	2.4	5.2	0.09	0.03	33	0.16	0.13	81	3.9	2.8	72
3	5	Leg muscle	73.3	54.4	3.7	7.9	0.11	0.02	18	0.15	0.14	93	5.6	2.9	55
2	8	Skin	48.5	17.9	46.7	61.7	0.03	0.01	33	0.07	0.06	86	3.8	3.9	100

[1] Determined by biological means; other thiamine values were determined by the thiochrome method. [2] All vitamin values for cooked meat are expressed in equivalents of fresh weights.

The apparent thiamine contents of breast and leg muscle and liver of Lot 1, determined by rat-growth, were usually greater than those of Lots 2 and 3, which were measured by thiochrome production. This discrepancy has often been noted. The riboflavin content of the tissues of Lot 1 also appeared greater than the contents of Lots 2 and 3, possibly due to the different enzyme treatment used in the extractions.

Losses of Vitamins in Frozen Storage: In none of the six tissues of Lots 2 and 3 stored for from three to nine months at −23°C.(−9°F.) could a definite trend toward loss of any of the vitamins be seen. Tissues of the stored birds as often as not yielded slightly larger vitamin values than those of freshly killed birds. This may have been due to the natural variation manifested by the limited sample, four to six turkeys in each lot, or to gradual enzymatic changes, allowing more complete release of vitamins after storage. In Lot 1 similar variability was found as a result of three months' storage, except that measurable losses of thiamine, 36 to 48 per cent, were found in breast and leg muscles, respectively. Thiamine values of Lot 1, found by the rat-growth method, agreed excellently with those of Lots 2 and 3 in the case of the stored samples, but were 40 to 60 per cent higher in the case of samples from freshly killed birds. This may have been due to the disappearance in storage of oxidizable fluorescent substances, which in the fresh samples were destroyed by the oxidation with ferricyanide, with resultant high blanks and low thiochrome readings.

TABLE 6

Riboflavin Assay of Turkey Breast and Leg Meat by Four Methods[1]

Sample	Solids	Rat-growth assay	Chick-growth assay[2]	Microbiological means	Fluorometric means
	pct.				
Breast meat	27.4	1.6	0.9	1.0	1.7
Breast, cooked	36.4	1.3	0.8	1.2	1.5
Leg meat	25.8	2.8	1.8	2.8	3.3
Leg meat, cooked	39.4	2.0	1.2	2.2	2.3

[1] Riboflavin in μg. per gm. fresh sample or equivalent. [2] The chick-growth assay was made by Dr. S. Lepkovsky, Poultry Division, Berkeley, Calif.

Losses of Vitamins in Cooking: Loss of thiamine was large during cooking of leg muscle and skin, from 67 to 82 per cent, but was less in breast muscle, 38 to 43 per cent (see Table 5). Riboflavin and niacin were less affected, with losses usually varying from 20 to 30 per cent. These losses may be compared with those found in chicken tissues, Morgan *et al.* (1949), cooked by broiling. Range of loss is similar, but generally greater, in the turkey meat. Loss of thiamine in leg meat, as well as of niacin in both breast and leg, was significantly greater in turkey than in chicken samples. These differences may be due to the marked differences in amount of heat exposure involved in methods of cooking chickens and turkeys.

One composite sample of breast muscle and one of leg muscle from Lot 1 were used to compare riboflavin determinations made by rat-growth, chick-growth, microbiological and fluorometric methods, and to note the apparent loss of riboflavin in cooking as shown by these assays (Table 6). Results obtained by rat-growth and fluorometry were comparable and the

yield was higher than in those obtained by chick-growth and growth of *Lactobacillus casei*. There was less discrepancy among values for riboflavin obtained by different methods in the case of cooked than of raw meat.

A somewhat similar comparison of riboflavin analyses was made earlier on prunes, Morgan, Bentley, and Groody (1944), and similar results obtained. The fluorometric and rat-growth methods yielded comparable and relatively large riboflavin values and the chick-growth and microbiological assays much lower values which were comparable with each other. Release of bound riboflavin, especially in the uncooked turkey samples, appeared to be more necessary for its utilization by chicks and microorganisms than by rats or fluorometry.

SUMMARY

Thiamine, riboflavin, and niacin content of breast muscle, leg muscle, liver, heart, gizzard, and skin was determined in three lots of Bronze strain turkeys, 64 birds in all. Some of each lot were examined immediately after killing, and some after three, five, six, or nine months' storage at —23°C. Samples of leg muscle, breast muscle, and liver were examined after cooking, as well as in the uncooked state.

Total loss of weight in cooking was always less in fresh than in stored birds, and less in larger than in smaller birds. Some off-flavors were detected in both fresh and stored turkeys fed rations containing 13 and 18.5 per cent fish meal, and rancidity was noted in several samples from birds fed tocopherol-free diets for several days before slaughter.

Vitamin distribution was similar to that found in chicken tissues. Thiamine and riboflavin were more concentrated in leg muscles, and niacin in breast muscles. Except for the liver and breast, which had 10 to 15 mg. per cent, all turkey tissues contained about the same amount of niacin, four to seven mg. per cent. Thiamine was more concentrated in the heart than in the liver. Riboflavin was found in greatest amount in the liver and heart, one to three mg. per cent; in leg muscle, 0.12 to 0.18; and in breast, 0.09 to 0.11.

No significant loss of vitamins occurred in three to nine months' storage at —23°C., except for thiamine in breast and leg muscles of one lot of turkeys.

Loss of thiamine in cooking was large, 67 to 82 per cent in leg and skin, 38 to 43 per cent in breast meat. Riboflavin and niacin suffered 20 to 30 per cent losses. Loss of vitamins due to cooking was greater in the roasted turkeys than in the broiled chickens previously studied.

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THE FREE AMINO ACIDS OF FRUITS

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The amino acids present in fruit tissues are believed to play a role in the non-enzymatic browning of fruit products, Joslyn (1941) and Stadtman (1948), and may be involved in enzymatic discoloration, Spitzer (1931). Spitzer (1931) reported the presence of an o-dihydroxyphenyl derivative in apples, pears, and other fruits, which he believed to be dopa. He reported that apples contained 0.1 per cent of dihydroxyphenylalanine, on the basis of colorimetric tests using a slightly alkaline solution of ferric chloride. The phenolase in these fruit tissues was found to react in a specific way with dopa but not with tyrosine. Since the occurrence of free amino acids and of dopa in fruit tissues has not been previously investigated, we have examined alcohol extracts of two varieties of apricots, two varieties of prunes, and one variety each of avocados and pears, for their content of amino acids by the partition chromatography technique described by Consden, Gordon, and Martin (1944).

EXPERIMENTAL PROCEDURE

Hard, green Moorpark apricots and firm, ripe Blenheim apricots from Santa Clara Valley; hard, green Newton Pippin apples from San Jose; green Imperial and French prunes from Sacramento; hard, ripe Fuerte avocados from Los Angeles County; and hard, ripe, yellowish Bartlett pears from Santa Clara Valley, harvested in August, were used. The greener fruits were selected, since these as a rule have a greater tendency to discolor and would likely have more dopa, if this were involved in discoloration. Extracts of these fruits were prepared several days after harvesting, the fruit being stored meanwhile at 0°C.(32°F.).

The apricots and prunes were halved and pitted; the apples and pears were cut into eighths, cored, and the stem and calyx removed; the avocados were halved, peeled, and pitted, using a stainless steel knife. Extracts from these tissues were prepared by blending 150-gm. portions with 700 ml. of 95-per cent alcohol in a Waring Blendor for five minutes. Assuming that the fruit contained on an average about 85 per cent moisture, this ratio of fruit tissue to alcohol would yield a mixture containing about 80 per cent of alcohol. At this level of alcohol content (70 to 80 per cent), Dent, Stepka, and Steward (1947) reported best extraction of free amino acids with the minimum of other contaminants. The extracts were allowed to stand overnight at room temperature, then shaken and stored at 0°C. for about one week before aliquots were taken for partition chromatography.

Stability of the cold alcohol extracts was not tested, but it is well recognized that even extracts obtained by use of boiling 80 per cent alcohol are stable only a few days at room temperature, although more

stable at 0°C. or below, Chemical Methods Committee (1935). Webster (1929, 1933), for example, reported deamination soon after cooling such extracts, but protein hydrolysis has not been observed. Since we were concerned primarily with the detection of amino acids present in higher concentration, extensive investigations of changes in the alcohol extracts were not made. Several observations made during storage of the alcoholic extracts at 0°C. indicate no significant changes in type or relative concentration of amino acids present. Enzymic hydrolysis during alcoholic extraction might have occurred, but the cut fruit was dropped rapidly into 95-per cent alcohol and immediately blended under conditions which usually are recognized to inhibit proteolysis.

Portions of the clear, supernatant liquid were used for chromatography. Aliquots of 200 λ,[1] representing about 42 mg. of fresh tissue, gave optimum separations on the chromatograms. Higher levels also were used, particularly in investigating the occurrence of dopa and detection of amino acids such as hydroxyproline. At levels of 400 λ, *i.e.*, 84 mg. of fresh tissue, the more intense spots tended to fuse with their neighbors. Furthermore, the relatively high concentration of other constituents (probably carbohydrates) at the origin began to interfere with normal development.

For purposes of hydrolysis, 200 ml. of extract were reduced to dryness in a lyophyll apparatus and the residue subsequently redissolved in 10 ml. of water; hence a 20-fold reduction of volume was achieved. Aliquots of 10 λ and 20 λ (42 and 84 mg. of tissue, respectively, on basis of fresh weight) of the concentrated solutions were hydrolyzed with five ml. 6N HCl at 15 pounds' pressure for six hours. After removal of water and HCl in an evacuated desiccator containing a tray of solid NaOH in addition to the desiccant, the residue was redissolved in the minimum amount of water and quantitatively transferred to the paper for chromatography.

In general, the selected aliquot of the clear extract of hydrolysate was applied to the filter paper (Whatman No. 1, 57 x 46 cm.) by means of a calibrated micropipette within a circle of one cm. radius, the center of which was located at a distance of six cm. from each of two adjacent sides of the rectangular sheet. When the aliquot was of such volume that a single application would have exceeded the boundary of the delimiting circle, successive portions were applied, each portion being permitted to dry in a current of air before application of the next. Thus, 10 applications of 20 λ each were required for the 200 λ aliquots.

Manipulative details of the technique were described adequately by Consden *et al.* (1944) and Dent (1948), hence need not be detailed here. Our procedures differed only in minor respects from the procedures of these authors. It has, however, been the experience of the junior author that a change in sequence of solvents may influence separation of the amino acids. Reasons for this are not entirely clear and seem to be manifold, *e.g.*, interfering substances in the paper itself or in the extracts. Most consistent results and best separations were obtained by employing water-saturated phenol as the developing solvent in the first direction; hence, this has been adopted as standard procedure throughout this work. Following

[1] $\lambda = 10^{-3}$ ml. = one microliter.

development of the chromatograms in water-saturated phenol, which under our conditions required from 18 to 24 hours, the papers were dried overnight in a current of air. Corresponding members of a duplicate set were then developed in the second direction in 2-4 lutidine-water (1:1 v/v) and in a butanol-acetic acid-water solvent of the following composition: four vol. n-butanol, one vol. glacial acetic acid, and five vol. water, Partridge (1946, 1948). The behavior of each spot was thus observed in three different solvents.

Identification of the resolved amino acids was accomplished by comparison of the relative position of the colored spots formed after development with ninhydrin; by the characteristic color of some of the spots; by observations made with individual amino-acids; and by noting the increase in intensity of a spot when a known amino acid was co-chromatographed with the extract. Asparagine, glycine, phenyl alanine, proline, dopa, and tyrosine form characteristic colored complexes with the ninhydrin reagent which assist in their identification; the color formed, however, varies with pH, but is constant for a given set of conditions.

Basis for identification is the difference in partition coefficients of the amino acids between the mobile organic phase and the stationary water phase, Consden *et al.* (1944). Identification becomes more certain when additional solvents are used; thus, it is improbable that an unknown ninhydrin-reacting substance would coincide in position with a known amino acid in all three solvent systems, unless the compounds were identical. The validity of this method is discussed in detail by Dent (1948) and others. It is unlikely that peptides would interfere with identification even if present, since their reactivity with ninhydrin is considerably less rapid. Hence, by observing rate of development of colored spots following application of ninhydrin to the paper, one obtains a clue as to whether a particular spot may be a peptide. It has been our experience that peptides do not, as a rule, occur in detectable amounts in the cold alcohol extracts of plant tissues.

Although Consden *et al.* (1944), reported that the lutidines were unsatisfactory solvents, in that they moved the amino acids too fast or unduly broadened the spots, one of us (Stepka) found by preliminary tests with standard solutions of the amino acids that a solvent composed of equal volumes of 2-4 lutidine [2] and water gives satisfactory separations without distortion of the spots. In fact, this solvent has certain advantages over the collidine-lutidine-water mixture employed by Dent, Stepka, and Steward (1947): *viz.*, (a) separations of the amino acids are equally good, (b) RF values are somewhat increased, and (c) the two-component system is less sensitive to temperature fluctuations than is the three-component system. Prepared in the proportions given, 2-4 lutidine and water form a homogeneous solution of one phase which does not separate into two phases at temperatures below 25°C.(77°F.); nevertheless, the lutidine is close enough to saturation with water to enable it to function in a partition chromatogram at normal working temperatures. It is possible that dif-

[2] Source: Koppers Company, Inc., Tar and Chemical Division, Pittsburgh 19, Pennsylvania.

ferent samples of 2-4 lutidine may require slightly different amounts of water to achieve a point of near-saturation, inasmuch as the commercial product is rarely pure. A simple distillation is generally effective in removing the colored components from the lutidine and it is the colorless distilled product which is used in this laboratory.

When butanol-acetic acid-water is used as the developing solvent in the second direction, the resulting chromatogram is somewhat more compressed and few of the amino acids change their relative positions as compared with the lutidine picture. Thus, glutamic acid travels farther than serine, alanine farther than threonine, and methionine overlaps valine. The overall picture is, however, satisfactory. Decomposition of cystine due to the high pH (*ca.* 12) of the lutidine solvent is retarded in the butanol-acetic acid-water solvent, to the extent that an initial application of 20 γ of this amino acid can be detected easily with ninhydrin.

The sensitivity of the method is given in tabulation of data for a number of amino acids by Dent (1948) and Pratt and Auclair (1948). For tyrosine, in which we are particularly interested, Dent gives 15 gamma for visual inspection and the latter workers give three gamma when viewed by transmitted light.

One of the objects of the present work was to determine, if possible, the presence or absence of dopa in the fruits under investigation. It thus became necessary to locate the position of dopa on the chromatogram. This was done by chromatographing aliquots of freshly prepared standard solutions of dopa, either alone, in combination with standard solutions of known amino acids, or as an addition to any one of the fruit extracts. Both the lutidine solvent and the butanol-acetic acid solvent were employed for development in the second direction.

It was observed that a long "tail" formed behind the dopa spot during the run in the lutidine solvent. This may be taken as evidence of decomposition during contact with the solvent. Further evidence of decomposition is obtained by comparing the intensity of the ninhydrin spot given by the same aliquot of the standard dopa solution on sheets chromatographed in the two solvents. In all such cases the color intensity of the dopa spot was considerably less on the lutidine papers than it was on the butanol-acetic acid chromatograms. Furthermore, the "tail" was eliminated in the latter solvent. Decomposition of dopa in the lutidine solvent is in accord with observations by Kisch (1930) that the spontaneous oxidation of dihydroxyphenylalanine is promoted by a decrease in the H-ion concentration, as well as by light or a rise in temperature. In the acidic solvent, applications of 20 γ of dopa at the origin produced distinct, easily identifiable spots. Dopa in concentration of the order of magnitude reported by Spitzer (1931) could easily be identified if present.

RESULTS AND DISCUSSION

Apples. The best re-solution of apple extract was found with 200 λ of extract. Asparagine, aspartic acid, and serine were the principal amino acids present. Spot No. 23 of Dent, Stepka, and Steward (1944) was also prominent. Dent (1948) has tentatively identified the latter as gamma

amino butyric acid. When this spot was checked against a sample of authentic gamma amino butyric acid,[3] the two exhibited identical behavior in all three solvent systems employed. This is strong evidence for the identity of the two. With higher levels of extract (300 to 400 λ) valine and leucine (or isoleucine) appeared as faint spots. There was no evidence of the occurrence of dopa or tyrosine.

Apricots. The two varieties of apricots used differed slightly in their amino acid content. In the Blenheim variety, at a level of 400 λ, alanine, asparagine, and aspartic acid occurred as principal amino acids with smaller amounts of serine, glutamine, glutamic acid, proline, valine, leucine (or isoleucine), and gamma amino butyric acid. No tyrosine or dopa was present. In the Moorpark variety, asparagine, aspartic acid, and glutamic acid occurred principally with smaller amounts of serine, glutamine, alanine, proline, valine, leucine (or isoleucine), and the gamma amino butyric acid. Neither tyrosine nor dopa was present. Chief differences between the two varieties were the noticeably lower amounts of alanine in the Moorpark and of glutamic acid in the Blenheim.

Pears. Of the fruits investigated, pears contained the smallest number of amino acids. At 400 λ, asparagine, serine, and glycine occurred principally with a trace of valine. The gamma amino butyric acid was not present, neither was dopa.

Prunes. The two varieties of prunes differed markedly in their amino acid distribution. The French variety contained asparagine and aspartic acid in larger amounts, and serine, glutamine, glutamic acid, and gamma amino butyric acid in smaller amounts at a level of 200 λ. When applied at the 400 λ level, proline and hydroxyproline were detectable. The Imperial variety at a level of 200 λ, contained asparagine, aspartic acid, and glutamic acid in principal amounts; also serine, threonine, alanine, glutamine, proline, valine, leucine (or isoleucine), and gamma amino butyric acid. At a level of 400 λ, hydroxyproline was revealed also. In neither variety was tyrosine or dopa detected. The Imperial variety contained more glutamic acid and in addition contained alanine, threonine, and valine.

Avocado. The avocado was richer in free amino acids than the other fruits tested. At 400 λ, asparagine, aspartic acid, glutamine, and glutamic acid were the principal amino acids. Serine, threonine, alanine, valine, and cystine occurred. Cystine was not positively identified. In addition, there were present gamma amino butyric acid and a spot tentatively identified as lysine.

Aliquots of the avocado extract were subjected to acid hydrolysis as described above. Under these conditions of hydrolysis, the amides, glutamine, and asparagine are converted into the corresponding amino acids. Increase in amounts of glutamic and aspartic acids [compare Plates 1(a) and 1(b)], as revealed by the intensity of the ninhydrin color produced following acid hydrolysis, is additional evidence for the correctness of the identification of the spots labeled glutamine and asparagine in Plate 1(a). It is to be noted that all spots identified in the unhydrolyzed extract (a)

[3] We are grateful to Dr. Dent for a generous sample of this material.

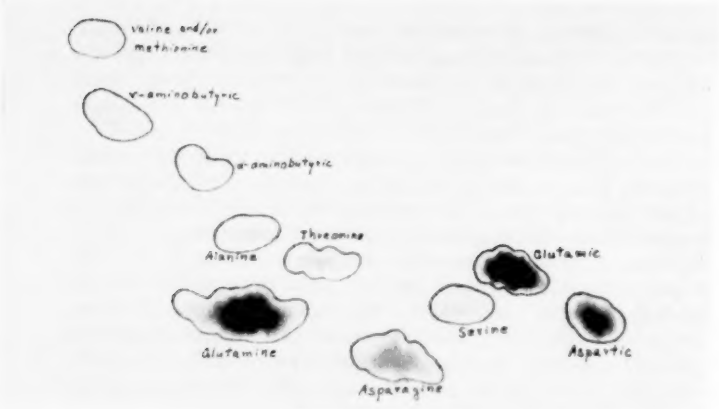

PLATE 1(a)
BuOH-HAc/H$_2$O
PHENOL

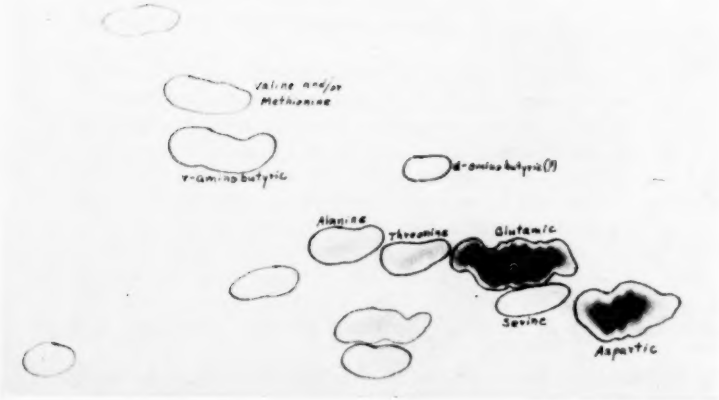

PLATE 1(b)
BuOH-HAc/H$_2$O
PHENOL

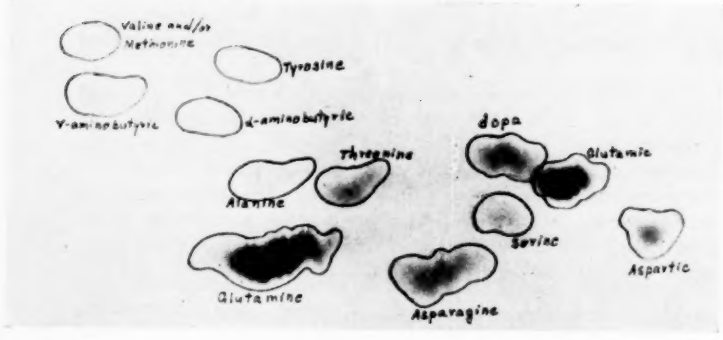

PLATE 1(c)
BuOH-HAc/H$_2$O
PHENOL

are accounted for in the hydrolyzed extract (b); hence, the occurrence of peptides at detectable levels in the unhydrolyzed extract is excluded.

The significant difference between the unhydrolyzed and the hydrolyzed samples is the disappearance of amides from the former and a corresponding increase of the respective dicarboxyllic amino acids in the latter. The hydrolyzed extract, Plate 1(b), shows a number of additional spots which are unidentified. These presumably are decomposition products of individual amino acids arising as a result of the hydrolytic treatment. The junior author has studied the effects of alkaline hydrolysis [$Ba(OH_2)$] on individual amino acids in connection with another study for which such information was desirable. It was found that chromatographically pure solutions of individual amino acids, *i.e.*, solutions known to yield only one spot on a two-dimensional chromatogram prior to hydrolysis, gave, when hydrolyzed, the characteristic spot for the particular amino acid, plus one to several additional ninhydrin-reacting spots. Thus, it is not improbable that some of the amino acids could yield similar decomposition products during the course of acid hydrolysis. This, of course, points to the exercise of great care in the interpretation of chromatograms of hydrolysates, as well as those of the soluble fractions. Plate 1(b) also shows the effect of residual HCl on the movement of the dicarboxyllic amino acids. Under conditions of low pH movement of these acids is accelerated, whereas the monoaminomonocarboxyllic acids are but slightly influenced, Consden *et al.* (1944). In chromatograms of the hydrolysate (b) glutamic and aspartic acids appear closer to serine than in the more neutral extract (a).

Plate 1(c) is included to show the position of dopa, if it were present. It is identical with (a) in every respect except that a solution containing a total of 20 γ of dopa was added to the aliquot of the fruit extract. Not only does the dopa spot appear at the same coordinates as it does when chromatographed alone but it also possesses its characteristic ninhydrin color. Accordingly, there can be little question about identity of the spot. No attempt was made to determine the limits at which dopa could be observed; however, a conservative estimate would be in the order of 5 γ, *i.e.*, a four-fold reduction of the amount used in (c). The tyrosine spot in (c) arises from the fact that it is present as a contaminant in our commercial sample of dopa. As previously stated, Pratt and Auclair (1948) give the limit of detection of tyrosine as 3 γ. The ninhydrin colors and sensitivities are very similar for both dopa and tyrosine.

PLATE 1. Photographs of chromatograms of Avocado Fuerte extract to show effect of hydrolysis (b) and position of dopa (c).

(a) Ten λ of unhydrolyzed concentrated extract, corresponding to 200 λ of the original extract. Note glutamine and asparagine spots.

(b) Ten λ of hydrolyzed concentrated extract, corresponding to 200 λ of original extract. Note disappearance of amide spots and concomitant increase in glutamic and aspartic acids.

(c) Identical with (a) in every respect except that 20 γ of dopa was added. Note position and intensity of dopa spot; compare with (a) to show non-occurrence of dopa in detectable amounts in original extract. Tyrosine spot is due to presence of tyrosine as contaminant in dopa sample.

Nonoccurrence of Dopa. In developing the chromatograms, particular attention was paid to dopa. The chromatograms were developed with both alkaline and acid solvents, with and without added dopa (Plate 1). The acid solvent was used to avoid possibility of oxidative destruction at higher pH levels, Kisch (1930). The dopa solution used was freshly prepared at a concentration of 1 γ per λ, and 20 λ of this solution gave a very distinct spot [Plate 1(e)]. If dopa were present in amounts of five to 10 gamma in the aliquots used for developing the chromatograms, it would have been detected. This amount would represent approximately 100th of one per cent on the basis of fresh weight of tissue. Consequently, if dopa does occur it follows it must be present in amounts below this value. In view of the fact that tyrosine, a possible precursor, also was not detected at any of the levels tested, it is doubtful that these low levels of dopa, if present, would be adequate to account for the observed browning. This indicates that contrary to Spitzer's (1931) belief, a specific dopa oxidase is not involved in the enzymatic browning of these fruits. Other evidence on this point is available in recent investigations on fruit phenolases.

SUMMARY AND CONCLUSIONS

The free amino acids present in the cold alcohol extracts, containing a final concentration of about 80 per cent alcohol, of apples, apricots, avocados, pears, and prunes were detected by partition chromatography. Avocados were found to contain most amino acids and pears least.

In all fruits examined, with exception of pears, there was present in addition to the previously known amino acids, an unknown amino acid which has been reported in a wide variety of animal and plant tissues and recently tentatively identified as gamma amino butyric acid, Dent (1948).

Hydroxyproline was found in prune extracts, but at low concentrations. This is the first reported occurrence of this amino acid in plant tissues.

Neither dopa nor tyrosine was found in the fruits tested at concentrations of over five to 10 gamma; hence, existence is questionable of a specific dopa oxidase functioning in discoloration of fruit tissues.

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DECREASE OF REDUCED ASCORBIC ACID IN GOAT'S MILK DURING STORAGE[1]

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Since goat's milk is consumed by a considerable number of Massachusetts residents, its vitamin C content is of interest from the standpoint of human nutrition. Gamble, Ellis, and Besley (1939), Turner and Garrison (1940), Richmond, Grinnells, and Satterfield (1942), Hunt and Winter (1922), Cimmino (1938), Holmes, Lindquist, and Greenwood (1945), Holmes, Lindquist, and Finnegan (1949), and others have shown that goat's milk contains a significant amount of reduced ascorbic acid (vitamin C).

Obviously, the ultimate consumer is interested, primarily, in the amount of reduced ascorbic acid present in the goat's milk when it is consumed. Gunsalus and Hand (1941), Hand (1943), Kothavalla and Gill (1943), Holmes and Jones (1945), and others found that the reduced ascorbic acid disappeared rapidly from cow's milk during the first few days after it was drawn from the cow. On the other hand, Holmes and Jones (1947) reported that reduced ascorbic acid disappears relatively slowly from mare's milk during the same period. A review of the literature revealed no information regarding the stability of reduced ascorbic acid in goat's milk when stored in the usual household type of refrigerator, and this study was undertaken to accumulate data concerning loss of reduced ascorbic acid from goat's milk.

EXPERIMENTAL PROCEDURE

Samples of goat's milk were obtained, during September, at the same evening milking from 16 different dairies in the Worcester, Massachusetts area. Samples from six of the dairies were individual animal samples. The remaining samples were pooled milk from two to nine goats. Ten samples came from Toggenburg goats, one from French Alpine, two from Saanen, and the other three from mixed herds of Saanen and Nubian, Toggenburg and Nubian, and Saanen and Toggenburg goats. Goats that produced the milk used in this study were of different ages, different stages of lactation and pregnancy, and were fed somewhat different rations. Accordingly, it was assumed that the samples represented so-called average commercial goat's milk produced in Massachusetts.

The milk was cooled as soon as possible after it was drawn from the goats. Then it was packed in ice and taken from the various dairies to the laboratory. In the meantime, the samples were not exposed to light or air. The milk was approximately 18 hours' old when the samples arrived at the laboratory. Assays of the raw goat's milk for reduced

[1] Contribution No. 713, Massachusetts Agricultural Experiment Station.

ascorbic acid were made immediately and subsequently at four 24-hour intervals. During the study the samples were stored in darkness at 10°C. (50°F.) in the bottles in which they arrived at the laboratory.

For comparison a sample of raw, pooled herd milk produced by Ayrshire, Guernsey, Holstein, Jersey, and Shorthorn cows was obtained on 17 consecutive Monday mornings. It was believed this milk was representative of high-grade, commercial cow's milk. The samples were assayed as soon as they arrived at the laboratory and at subsequent 24-hour intervals. The cow's milk samples were stored under the same conditions and in same refrigerator used for the goat milk samples. Also, the first reduced ascorbic acid assay of the cow's milk was made when the samples of milk were of the same age as that of the goat's milk.

Obviously, both the goat's and cow's milk contained a larger amount of reduced ascorbic acid when the milk was drawn from the animals, but this study was made to determine amount and rate of loss of reduced ascorbic acid from commercial goat's milk during the period it might be in the ultimate consumer's possession. The 80 assays of goat's milk for reduced ascorbic acid were made in accordance with the procedure suggested by Sharp (1938), except 25 ml. instead of 10 ml. of milk were used, and a mixture of eight per cent acetic and three per cent metaphosphoric acids was used in place of sulfuric acid.

RESULTS AND DISCUSSION

Average values for reduced ascorbic acid obtained for the samples of goat's milk and cow's milk at each of the five assay periods are plotted as curves (Chart 1). Amounts of reduced ascorbic acid in goat's milk and cow's milk at the beginning of the study were essentially the same, being 16.8 and 17.4 mg. per liter, respectively.

The goat's milk lost 31.5 per cent of its reduced ascorbic acid during the first 24 hours of storage. Rate of loss on the second and third days was much less than for the first day, being 8.3 and 8.9 per cent, respectively, and 12.5 per cent was lost during the fourth day. Thus, total loss of reduced ascorbic acid for goat's milk stored four days in a home-type refrigerator, in darkness at 10°C., was 61.2 per cent. Average amount of reduced ascorbic acid in the cow's milk at the beginning of the study was 17.4 mg. per liter, and at the end of the study it was 6.0 mg. per liter. Accordingly, during the four-day storage period under the same conditions as were employed for the goat's milk, the cow's milk lost 65.5 per cent of the reduced ascorbic acid that it contained at the beginning of the study.

Values obtained in this instance for reduced ascorbic acid loss from goat's milk and from cow's milk, 61.2 and 65.5 per cent, respectively, are in close agreement with those reported by Gunsalus and Hand (1941), who found a decrease from 14.9 mg. of reduced ascorbic acid per liter at the beginning of storage, to 5.1 mg., or a 65.1 per cent loss during four days' storage of cow's milk. On the other hand, they are higher than those observed by Hand (1943), who noted a loss of from 19.0 mg. of reduced ascorbic acid per liter to 10.9 mg., or 42.7 per cent during a similar period of storage of cow's milk at 1°C.(32°F.). However, all these values are quite different from those obtained by Holmes and Jones (1947) for

stability of reduced ascorbic acid in mare's milk; i.e., during four days' storage at 10°C. the average amount of reduced ascorbic acid in eight samples dropped from 121 to 114 mg. per liter, a loss of 5.8 per cent. Thus, data accumulated in this study seem to show that rate of loss of reduced ascorbic acid from the milk of two species of ruminant herbivora, goats and cows, is approximately identical.

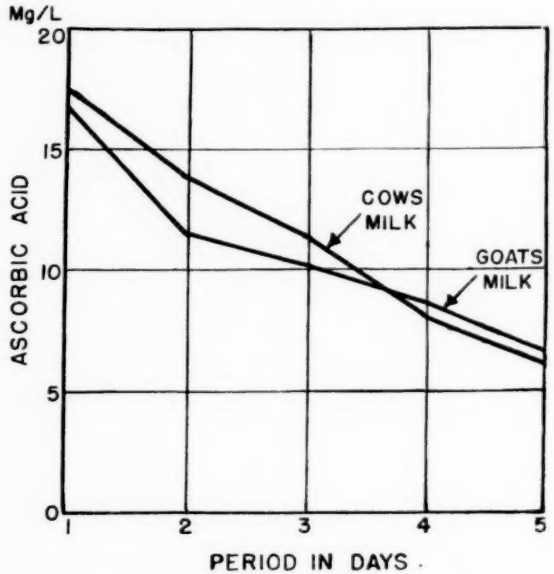

CHART 1. Loss of reduced ascorbic acid during storage.

SUMMARY

Samples of milk were obtained from representative goat dairies in central Massachusetts. On arrival at the laboratory and at four subsequent 24-hour intervals they were assayed for reduced ascorbic acid. On the average the goat's milk lost 31.5, 8.3, 8.9, and 12.5 per cent of reduced ascorbic acid, respectively, per day, or a total of 61.2 per cent during four days' storage in darkness at 10°C. Samples of cow's milk stored under the same conditions lost 65.5 per cent. The data suggest a similarity of conditions in which the reduced ascorbic acid exists in the milk of the two species of ruminants.

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CHEMICAL STUDIES ON GRAPES AND WINES. I. VOLATILE CONSTITUENTS OF ZINFANDEL GRAPES (*VITIS VINIFERA*, VAR. ZINFANDEL)[1]

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In an attempt to acquire information on the chemical processes involved in wine production, it is essential to study the chemical composition of both the starting material, the grapes, and the finished product, the wine. The following investigation is concerned with the volatile material present in Zinfandel grapes and will be followed by a study of the non-volatile constituents.

Our knowledge of the chemical composition of grapes is limited, for the most part, to estimation of the non-volatile constituents, such as acids, sugars, proteins, etc., without a more accurate determination of the exact chemical nature of the compounds.

However, as early as 1891, glyoxylic acid was found in grapes by Ordonneau (1891) and his finding later confirmed by Semichon and Flanzy (1933). Power and Chestnut (1921), Scott (1923), and Sale and Wilson (1926) have found methyl anthranilate in grape juice of the variety *vitis labrusca*, in amounts varying from zero to 3.80 mg. per liter. Sale and Wilson (1926) failed to find this ester in the fruit of *vitis vinifera*, although they reported the finding of six to 366 mg. of volatile ester and three to 12 mg. of volatile acid per kg. of grape juice in a number of varieties of grapes. The presence of ethyl alcohol in the amount of 0.4 to 4.70 per cent by volume in grape juice concentrate was reported by Fabre and Bremond (1933).

In 1943 Sanchez Pachas also reported the finding of ethyl alcohol in pure grape beverage in an amount of 5.8 to 16.5 gm. per liter. Both authors reported without trying to differentiate whether it was present originally as a natural constituent or as a fermentation product. Sanchez Pachas (1943) also determined the volatile acid content of grape juice and expressed his result collectively as acetic acid (0.328 to 1.37 gm./kg.).

The native American grape, *vitis labrusca*, on account of its marked fragrance, is cultivated chiefly for table use and for the production of beverages, while *vitis vinifera*, although less fragrant, has a higher sugar and total solid content, and is therefore commonly used for the making of raisins and for the production of wine. In California, *vitis vinifera* var. Zinfandel is very extensively used for wine-making and has been thor-

[1] This investigation was supported by a grant from the State of California Wine Advisory Board.

oughly studied by viticulturists. It was, therefore, selected as the subject of this investigation.

In our investigation, the volatile material was distilled off in vacuum at 40 to 50 mm. of Hg. and the volatile material condensed in a series of traps maintained at low temperature. This distillate, mostly water, containing very small amounts of volatile materials, was refractionated and concentrated at reduced pressure, and finally separated from the water by ether extraction, after saturation with ammonium sulfate.

The volatile oil obtained from 1,500 kg. of de-stemmed fruit amounted to 375 gm. This material was fractionated and the several fractions individually analyzed for their constituents, and if necessary, hydrolyzed. Hydrolysis of the oil was carried out after the fractionation, since it was desirable to know the composition of the individual esters, if present. Identities of the compounds were established by preparation of appropriate derivatives; i.e., dinitrobenzoates for alcohols, p-phenylphenacyl esters for the acids, and 2,4-dinitrophenylhydrazones for the carbonyl compounds.

The following table gives the approximate amounts of compounds identified in terms of gm. per 1,000 kg. of de-stemmed fruit (Table 1). It should be pointed out that the presence of sulfur may be explained by the application of chemical sprays used during the cultivation of grapes.

TABLE 1

Summary of Components of the Volatile Oil of Zinfandel Grapes

	gm./1,000 kg. fruit
Acetaldehyde	1.800
Leaf aldehyde	0.327
Ethyl alcohol	244.000
Acetyl methyl carbinol	0.013
Acetic acid	0.0053
n-Butyric acid	0.003
n-Caproic acid	0.0015
Glyoxylic acid	0.118
n-Butylphthalate	2.250
Waxy substance	0.024
Carbonyl compound	0.025
Sulfur	0.004

EXPERIMENTAL PROCEDURE

Fresh grapes of the Zinfandel variety were supplied by the Fruit Industries of Guasti, California. Four thousand pounds of Zinfandel grapes, after being washed and de-stemmed by hand, were passed through a Sepra-Sieve. The 1,100 liters of juice collected were distilled in a vacuum of 40 to 50 mm. at a temperature of 45 to 50° C. (113 to 122° F.). Samples of the distillation residue, as well as pulp and seeds from the grapes, were saved for future analysis.

Distillation apparatus used was part of the equipment of the U.S.D.A. Laboratory of Fruit and Vegetable Chemistry in Los Angeles. It was a flash concentrator of stainless steel construction, and was of a convection circulating type. It was equipped to be operated under vacuum. In

addition to the regular condensate receivers located after a double water condenser, there was also a two-liter flask located after a refrigerated condenser cooled to approximately 2°C. with glycol coolant. These traps were followed by a series of one large and three small dry-ice/alcohol traps, which preceded the high vacuum pump.

The distillate recovered from the concentrator, amounting to 440 liters, was passed through a continuous stripping column, at reduced pressure, to give a concentrate distilling over at 45 to 50° at 40 to 50 mm. pressure.

The oil-aqueous concentrate, plus the materials collected in a series of four traps, amounted to 37.15 liters. This was saturated with c.p. ammonium sulfate and extracted with peroxide-free ether. The ether extract, after being dried over anhydrous sodium sulfate, was distilled to remove the ether. The ether-free liquid, amounting to 158.3 gm., was fractionated by using a 12-inch Vigreux column, first under atmospheric pressure and then at reduced pressure, into the following fractions (Table 2).

TABLE 2

Fractionation of 158 gm. of Volatile Oil from 1,500 kg. of De-stemmed Fruit

Fraction No.	Distillation temp.	Pressure	Fraction wt.
	°C.	mm.	gm.
1	34.0-39.0	740	5.5
2	39.0-49.0	740	18.0
3	49.0-59.0	740	4.6
4	59.0-69.0	740	3.8
5	69.0-76.5	740	15.5
6	76.5-76.8	740	19.2
7	76.8-77.0	740	84.2
8	77.0-80.0	740	0.3
9	80.0-84.2	740	1.4
10	84.2-85.2	740	3.7
11	85.2-87.2	740	0.5
12	87.2-91.5	740	0.5
13	91.5-93.0	740	0.5
14	93.0-95.0	740	0.5

Distillation was interrupted at this point and the residue extracted with two per cent sodium carbonate solution; the alkali insoluble residue was taken up with peroxide-free ether, washed once with water, then dried over anhydrous sodium sulfate. Fractional distillation was resumed at reduced pressure and five additional fractions collected (Table 3).

TABLE 3

Fractionation Under Reduced Pressure

Fraction No.	Distillation temp.	Pressure	Fraction wt.
	°C.	mm.	gm.
15	101-135	0.015	0.10
16	135-170	0.015	0.22
17	170-190	0.010	3.50
18	195-200	0.010	0.30
19	Boiler residue	.....	0.40

Since Fraction 17 covered a large boiling point range with considerable quantity of distillate, it was further fractionated at reduced pressure into four fractions (Table 4).

TABLE 4

Refractionation of Fraction 17

Fraction No.	Distillation temp.	Pressure	Fraction wt.
	°C.	mm.	gm.
20	195-200	0.05	1.36
21	200-205	0.05	1.59
22	Boiler residue	.....	0.28
23	.....[1]	.....	0.25

[1] Liquid recovered from dry ice/acetone trap during distillation.

Study of Aqueous Phase From Ether Extraction: The aqueous residue (37.15 liters) from the ether extraction still gave a positive test for aldehyde with the Fuchsin-aldehyde reagent; hence, it was redistilled, using a 22-liter Pyrex balloon flask. Distillation was carried out at a pressure of 21 mm. of mercury with the still contents being kept below 50°C. at all times. The vapor passed through a spiral glass condenser into a flask cooled by ice, then through a flask immersed in an ice-salt bath, and finally into a bath cooled by dry ice/acetone. Condensates in the ice and ice-salt baths amounted to 1.5 liters, gave a negative test for aldehyde with the Fuchsin-aldehyde reagent, and gave a positive iodoform test for alcohol. The contents of the dry ice/acetone trap (120 ml.) gave a positive Fuchsin-aldehyde test. Two one-ml. portions of this liquid were taken out and derivatives of p-nitrophenylhydrazine and dimethyldihydroresorcinol prepared. The remainder of liquid was quantitatively converted into a 2,4-dinitrophenylhydrazone by the method of Iddles and Jackson (1934).

The dry ice/acetone trap material produced 13.8 gm. of crystalline 2,4-dinitrophenylhydrazone which melted at 163 to 166°C.(325 to 331°F.). After recrystallization three times from ethyl alcohol and water, it melted at 165 to 166°C.(329 to 331°F.) (Table 5).

TABLE 5

Characterization of Acetaldehyde

Derivative	Der. dry ice/ acetone trap	Der. Synthetic	Mixed
	m.p.°C.	m.p.°C.	m.p.°C.
2,4-Dinitrophenylhydrazone	165 -166	165-166	165-166
p-Nitrophenylhydrazone	129.5-130	129	129
Dimedone	146.0-146.5	145-146	145-145.5

Total amount of acetaldehyde present in the dry ice/acetone trap was 2.7 gm., as calculated from weight of the derivatives.

After aldehyde was removed, the contents of traps were combined and fractionally distilled. The distillate, amounting to 490 ml., was collected. The distillate was identified as ethyl alcohol by its physical properties as well as by the preparation of its derivative, 3,5-dinitrobenzoate.

Total amount of alcohol in the distillate was estimated by determining the refractive index after diluting with three times its volume of water. The refractive index found (n_D^{25}) was 1.3390, which indicated the presence of 72.1 per cent by volume of alcohol in the 490 ml. of original distillate. It is equivalent to 280.4 gm. pure alcohol.

The derivative, 3,5-dinitrobenzoate, melted at 92.5 to 93°C. (198 to 199°F.) and did not depress the melting point of an authentic sample of ethyl 3,5-dinitrobenzoate.

Identification of Fractions 1, 2, 3, and 4: Fractions 1 through 4 were found to be mixtures of ether and ethyl alcohol.

Identification of Fractions 5, 6, and 7: Fractions 5, 6, and 7 were shown by their physical properties to be ethyl alcohol. They were further characterized by the preparation of a 3,5-dinitrobenzoate which melted at 92 to 93°C. (197 to 199°F.). It gave no depression in mixed melting point with a known sample of ethyl 3,5-dinitrobenzoate.

Identification of Fractions 8, 9, 10, and 11: These fractions consisted of alcohol and traces of components of higher boiling point. They gave a faint aldehyde reaction with Fuchsin-aldehyde reagent. The aldehyde was removed quantitatively by conversion into a 2,4-dinitrophenylhydrazone, which was purified by passage through a chromatographic column by the method of Roberts and Green (1946). By this method two bands were obtained. From the lower band was recovered a 2,4-dinitrophenylhydrazone which melted at 118°C. (244°F.). Microchemical analysis gave 23.13 per cent of nitrogen, 45.42 per cent of carbon, and 4.17 per cent of hydrogen. Due to lack of material, further identification could not be made.

An orange band was formed near the top of the column. The orange band was cut off and eluted with 1:1 ethanol-ether solution, from which leaf aldehyde was identified as follows:

Anal. of 2,4-dinitrophenylhydrazone, m.p. 144°C.:
Calcd. for $C_{12}H_{14}N_4O_4$: C, 51.80; H, 5.03; N, 20.05.
Found: C, 51.53; H, 5.07; N, 20.05.

The solvent was distilled off the light-yellow solution which passed through the column and a colorless crystalline substance obtained after several recrystallizations from 95 per cent ethyl alcohol. It melts at 51 to 52°C. (124 to 126°F.). Microchemical analysis gave 83.19 per cent of carbon, 14.16 per cent of hydrogen, and a molecular weight of 338.6. These data gave an empirical formula to $C_{24}H_{50}$.

After the removal of aldehyde, the filtrate was distilled and identified as ethyl alcohol by preparation of a 2,5-dinitrobenzoate which melted at 92 to 93°C. and gave no mixed melting point depression with a known compound.

Identification of Fractions 20 and 21: Microchemical analysis of Fractions 20 and 21 indicates they are identical. Since this component gave negative tests for acid, alcohol, and carbonyl compound, it is probably an ester. This fraction was saponified with excess sodium hydroxide, which gave a saponification equivalent of 134. The volatile alcohol formed by saponification of ester was distilled and identified as its 3,5-dinitrobenzo-

ate derivative. The 3,5-dinitrobenzoate, which melted at 65°C.(149°F.) gave no depression of mixed melting point with the derivative of synthetic n-butyl alcohol (Table 6).

TABLE 6

Physical Properties and Microchemical Analysis of Fractions 20 and 21

Fraction No.	Density	Refractive index	Sap. Eq.	M.W.	C	H
					pct.	*pct.*
20	.....	.....	.....	.....	66.33	8.35
					66.14	8.39
21	d_4^{25} 1.0543	n_D^{25} 1.4850	134	314.00	66.67	8.23
Calcd. for $C_8H_{22}O_8$	.....	.....	.....	.....	69.04	7.97
Reported in literature [1]	d_{20}^{20} 1.047	n_D^{20} 1.4900	139	278.34	.....	.....

[1] Huntress and Mulliken, 1946.

The residue after distillation of alcohol was acidified with 6N sulfuric acid and extracted with peroxide-free ether in a continuous extractor. After removal of the ether, a white crystalline solid was obtained. After several recrystallizations from alcohol and water, it melted at 218 to 223°C.(424 to 433°F.) by slow heating or at 225°C.(437°F.) by rapid heating. Both types of melting were accompanied by decomposition. In a sealed tube it melted at 190°C.(374°F.). Microchemical analysis and conversion of the compound into phthalic anhydride by heating with a little acetic anhydride proved it to be phthalic acid. The phthalic anhydride obtained sublimed into characteristic long needles which melted at 130°C.(266°F.) in a sealed tube.

Anal. of free acid from saponification:
Calcd. for $C_8H_6O_4$: C, 57.83; H, 3.64.
Found: C, 58.04; H, 3.89.

Identification of Fraction 23: Fuchsin-aldehyde reaction indicated presence of aldehyde. A 2,4-dinitrophenylhydrazone was prepared and, after purification in a chromatographic column, melted at 145°C.(293°F.).

Anal. of 2,4-dinitrophenylhydrazone:
Calcd. for $C_{12}H_{14}N_4O_4$: C, 51.80; H, 5.03; N, 20.05.
Found: C, 51.96; H, 5.44; N, 20.36.

Mixed melting point with leaf aldehyde 2,4-dinitrophenylhydrazone isolated from Fraction 10 did not give depression.

Identification of Acidic Components: The sodium carbonate soluble aqueous portion was acidified with 6N sulfuric acid and extracted with peroxide-free ether in a continuous extractor. The ether was distilled off. To the aqueous residue was added two per cent sodium bicarbonate solution and the solution immediately extracted with peroxide-free ether.

The sodium bicarbonate soluble portion (255 mg.) of the acidic components was converted quantitatively into the p-phenylphenacyl ester which

was then purified by the chromatographic separation methods of Kirchner *et al.* (1946).

By this method the following esters were obtained (Table 7).

TABLE 7

Characterization of Sodium Bicarbonate Soluble Acidic Components

Band No. from top of column [1]	p-Phenylphenacyl ester	Synthetic p-phenylphenacyl ester	Mixed
	m.p.°C.	*m.p.°C.*	*m.p.°C.*
1	114-115	115 Acetic acid	114-115
2	85	85-86 n-Butyric acid	85-85.5
5	69-70	70-71 n-Caproic acid	70-71
9	132-133	132-133 Glyoxylic acid	132-133
10	52-53[2]	.....	.....

[1] Band numbers not shown were either highly fluorescent impurities or too small to be identified. [2] A crystalline substance isolated from the solution went through the column during development. Due to insufficient amount, further identification was not made.

Band No. 9 was further characterized by michrochemical analysis as follows:

Anal. of Band 9, p-phenylphenacyl ester:

Calcd. for $C_{16}H_{12}O_4$: C, 71.63; H, 4.51.

Found: C, 71.85; H, 4.86.

The sodium bicarbonate insoluble material (1.097 gm.) was distilled in a three-ml. Claisen flask and separated into four fractions (Table 8).

TABLE 8

Fractionation of Sodium Bicarbonate Insoluble Acidic Components

Fraction No.	Distillation temp.	Pressure	Fraction wt.
	°C.	*mm.*	*gm.*
1	35-45	750	0.240
2	45-130	750	0.390
3	135-160	.001	0.424
4	Boiler residue	.....	.....

Fraction 1 was found to be ether.

Fraction 2 gave a positive test for carbonyl group with 2,4-dinitrophenylhydrazine. The carbonyl compound was removed quantitatively by conversion to 2,4-dinitrophenylhydrazone. The mother liquor was distilled and gave a positive iodoform reaction for alcohol. It was further characterized by preparation of a 3,5-dinitrobenzoate derivative which melted at 93°C.(199°F.). It gave no mixed melting point depression with a known sample of ethyl 3,5-dinitrobenzoate.

The odor of the carbonyl compound which was previously noted suggested this compound might be acetylmethyl carbinol. This was further confirmed by microchemical analysis.

Anal. of 2,4-dinitrophenylhydrazone, m.p. 315°C. decomp.:

Calcd. for $C_{16}H_{14}N_8O_8$: C, 43.06; H, 3.16; N, 25.11.

Found: C, 43.65; H, 3.61; N, 25.10.

Mixed melting point with a known sample af acetylmethyl carbinol 2,4-dinitrophenylhydrazone gave no depression.

Fraction 3: Upon alkaline hydrolysis the alcohol and the acid formed by hydrolysis were isolated and identified as n-butyl alcohol and phthalic acid, respectively, by procedures described previously. Hence, this fraction was n-butyl phthalate.

Fraction 4: This fraction upon standing gave a deposit of some crystals. They were separated and purified by recrystallization from ethyl acetate. The crystals melted at 118 to 123°C.(244 to 253°F.) and microchemical analysis revealed the absence of carbon, thus indicating an inorganic compound. The physical properties suggested sulfur, and this was confirmed by the following tests:

1) Solubility in carbon disulfide. 2) Conversion into sulfide by fusion with metallic sodium and subsequent precipitation as lead sulfide with soluble lead acetate in dilute acetic acid solution. The lead sulfide was further confirmed by conversion into white lead sulfate by the addition of three per cent hydrogen peroxide. 3) Mixed melting point with an authentic sample previously recrystallized from ethyl acetate gave no depression.

SUMMARY

The chemical composition of the volatile oil from fresh Zinfandel grapes has been investigated. The following compounds were found to be present: ethyl alcohol, acetaldehyde acetic acid, n-butyric acid, n-caproic acid, glyoxylic acid, n-butyl phthalate, leaf aldehyde, sulfur, and acetyl-methyl-carbinol.

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CONSERVING VITAMIN C BY VARYING CANNING PROCEDURES IN SNAP BEANS, TOMATOES, PEACHES, AND PEARS

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This research was undertaken to determine procedures whereby processed foods could make a greater contribution in vitamin C than had already been reached by usual methods of canning.

A survey on canning practices in Wyoming rural homes revealed that tomatoes, snap beans, peaches, and pears were canned in larger quantities than other foods. Therefore, this study was confined to an investigation of their contribution in vitamin C as ordinarily canned in the home, and as canned by changes in technique or by enforcement with synthetic ascorbic acid.

Surveys have shown that vitamin C is frequently low in dietaries during winter months, particularly in those rural areas where distances to towns are great and fresh vegetables and fruits are scarce at the markets. Many families in isolated areas depend upon processed foods as the chief source of vitamins and minerals during winter months.

A canning-practices survey in Wyoming shows that the amount of the home-processed pack of snap beans ranks second-high and next to that of tomatoes, and that large quantities of both are canned. Practically all varieties of snap beans may be grown at the lower elevations in Wyoming; in certain areas they are an important crop for commercial canning.

The earlier-maturing varieties of peaches and pears have a low natural vitamin C content. After canning, this nutrient was almost *nil* in pears and very low in peaches. An investigation of methods for conservation of ascorbic acid in canning either fruit was therefore not warranted. Addition of powdered l-ascorbic acid to both pears and peaches, and its effect upon increasing vitamin C, as well as improvement in the quality, were investigated.

Tomatoes used in these canning tests were from a hybrid variety crossed with the early-maturing Bounty, and were developed at the Federal Horticultural Station at Cheyenne, Wyoming, where the altitude is about 6,000 feet. (Tomatoes do not ripen in the cool climates and short summers of the 7,200-foot elevation of the Agricultural Experiment Station at Laramie, Wyoming.) At the Cheyenne station the vines were pole-supported, thereby exposing the tomatoes to more sunshine than if they had been on unsupported vines. The tomatoes were shipped from Cheyenne into Laramie (about 50 miles distant) by express and analyzed for vitamin C about 18 hours after harvest. They were refrigerated when received at the laboratory.

REVIEW OF LITERATURE

Survey of the literature reveals few investigations on retention of vitamins in home-canned foods, but a considerable number on those commercially canned.

Cameron (1949) points out that the present research of the Joint Canners Association and of the Can Manufacturers Institute is accumulating accurate information on nutritive values of commercially canned foods. Their average ascorbic acid values for the same commodities tested in the Wyoming study were 3.2 mg./100 gm. for snap beans, 16.5 for canned tomatoes, 3.9 for clingstone peaches, and 1.5 for pears.

Hunt, Sunnell, and Pfaff (1944) reported that most of the vitamin C lost in canning was due not to actual processing, if properly carried out, but to such other conditions as blanching and to the amount of headspace in the can containing air. Tin cans entrapped less air than glass, with their dome-shaped closures.

Mayfield and Richardson (1939) and Farrell and Fellers (1941) reported high ascorbic-acid losses in home-canned snap beans ranging from 75 to 85 per cent of the initial value. Mayfield and Richardson (1939) gave values of three to six mg./100 gm. for the home-canned and 27 mg./100 gm. for the raw snap beans. Eheart and Sholes (1948) noted that bean pods given a "French" cut lost more than those snapped across. Lamb, Pressley, and Zuch (1947) observed that blanching accounted for the principal loss of water-soluble C at commercial canneries, but that a considerable amount was lost between blanching and processing. Actual processing itself did not involve great losses if the air entrapped was not excessive. Their canned values were 3.4 mg./100 gm.

Data reported by a number of investigators indicate that the initial value of ascorbic acid in vegetables and fruit will vary with variety and growing conditions.

Hayden, Heinze, and Wade (1948) compared over 200 strains and varieties of snap beans and found those highest in ascorbic acid had approximately twice as much as the lowest. Average values for the raw beans were 22.8 mg./100 gm. on a fresh-weight basis.

Mituda (1938) and Hamner, Bernstein, and Maynard (1945) reported that the amount of vitamin C in certain fruits including tomatoes, and in certain vegetables, was proportional to the amount of sunlight received during ripening.

In this connection Brown and Moser (1941) and Clayton, Pressey, and Lees (1948) reported that tomatoes grown on pole-supported vines, in which there was greater exposure to the sun, had more vitamin C than those on unsupported vines, where they were shaded by the leaves.

Leverton, Peters, and Schlaphoff (1945) pointed out that blanching tomatoes for 20, 30, and 60 seconds caused a decrease in ascorbic acid of seven, 16, and 23 per cent, respectively. Processing in the jars caused an additional four per cent loss; nine months of storage in pint jars caused 47 per cent more.

Patton (1946) concluded that loss of vitamin C in home-canned tomatoes was moderately rapid for the first three months of storage. Retention at the end of one year was slightly more than one-half the amount they had immediately after canning.

Mitchell and Roderick (1945) reported that ascorbic-acid values ranged from 4.03 to 9.6 mg./100 gm. in South Carolina peaches and varied with the variety. Elberta peaches contained 4.03 mg./100 gm. before peeling,

3.74 after peeling, and 3.54 after processing. Lamb, Pressey, and Zuch (1947) reported eight mg./100 gm. for freestone peaches and 15.5 for clingstone. Wiegand (1946) found that action of oxidase in processed peaches may cause off-flavors, but that addition of ascorbic acid retarded the enzyme-catalyzed oxidative changes. Powers and Fellers (1945) also noted quality improvement in certain canned fruit by enforcement with ascorbic acid. Bauernfeind, Batcher, and Shaw (1947) suggested that a possible reason for improvement was that ascorbic acid reacts with the oxygen left in the canned fruit, thereby protecting the fruit from oxidation.

EXPERIMENTAL PROCEDURE

Canning Snap Beans: The Stringless Green Pod variety of snap bean was processed within a few hours after harvest. The fresh beans were graded and pods of the same degree of maturity selected for processing.

Manipulative procedures in canning the snap beans were varied, as described below:

Lot 1: The pods were cut into one-inch pieces, blanched by precooking in boiling water five minutes in an open kettle, packed into No. 2 tin cans, covered with boiling fresh brine, sealed, and processed 25 minutes at 116°C.(240°F.).

Lot 2: Pods were cut across into one-fourth-inch pieces and treated as above.

Lot 3: Pods were given a "French" cut by cutting into thin strips lengthwise, then treated as above.

Lot 4: Pods were cut into one-inch pieces, packed raw into cans, and covered with boiling brine. The cans were exhausted of air and processed 30 minutes at 116°C. (240°F.).

Lot 5: Pods were cut across into one-fourth-inch pieces and treated as in Lot 4.

Lot 6: Pods were given a "French" cut by cutting into thin strips lengthwise, then treated as in Lot 4.

In the lots where a precook was preliminary procedure before packing, the technique consisted in putting washed pods quickly into a measured quantity of boiling water—sufficient to cover them—and boiling in an open kettle five minutes. Other lots were enforced with powdered l-ascorbic acid in amounts of 200 mg.

Equal weights of beans and the same measure of liquid were used for the different lots which were packed into No. 2 tin cans. After processing was completed, the tin cans were cooled in running tap water to about 16°C.(60°F.).

Canning Tomatoes: The tomatoes were canned in glass pint jars. The fruit—dipped into boiling water to loosen the skin—was peeled, packed, and processed as outlined below:

Lot 1: The peeled tomatoes were left whole and packed raw into the cans, pressed down enough to fill spaces, exhausted, and processed 50 minutes in a boiling water bath.

Lot 2: They were quartered, packed raw into jars as in Lot 1, and also processed 50 minutes. The time was lengthened over that recommended for sea level to compensate for the lower temperatures of boiling water at the 7,200-foot elevation, 93°C. (199°F.).

Lot 3: The peeled tomatoes were left whole, but boiled three minutes in tomato juice in an open kettle. They were packed into jars while hot and processed 20 minutes in a boiling water bath.

Lot 4: The tomatoes were quartered and boiled two minutes, then packed hot and processed as in Lot 3.

Lot 5: The tomatoes were simmered, sieved, filled hot into cans, and processed 15 minutes in a boiling water bath.

Canning Peaches and Pears: The peaches were dipped into boiling water to loosen the skins for peeling. The peeled fruit was dropped in a two-per cent citric-acid bath to safeguard against darkening before packing. The peaches were halved and the pears quartered and packed raw into hot, sterilized-glass pint jars. The fruit was covered with a 40-per cent hot sugar syrup to which powdered ascorbic acid was added in proportions ranging from 200 to 500 mg. per pint jar. The raw packed peaches were processed 35 minutes and the pears 50 minutes. Time for processing was lengthened over that at low altitudes, to compensate for the lower temperature of boiling water.

Ascorbic acid used for fortifying the fruit was Grade U.S.P. from Merck. The amount to be used was weighed out on an analytical balance and dissolved in a small amount of warm water before adding to the syrup. It was poured over the fruit just before processing. The jars of beans and fruit were stored in a cool, dark basement where temperatures ranged from 4 to 16°C.(40 to 60°F.). They were tested for quality and retention of ascorbic acid after from six to seven months.

Extraction of Ascorbic Acid: Extraction of ascorbic acid was made with one per cent metaphosphoric acid, and decolorization of the indophenol dye measured by an Evelyn photoelectric colorimeter, according to the method of Loeffler and Ponting (1942). Ascorbic-acid analysis on the fresh beans was completed on the day harvested. The fresh tomatoes were analyzed about 18 hours after harvest.

In sampling the raw tomatoes for ascorbic acid, a complete sample was made up of sectors from each of three unpeeled tomatoes. Duplicate samples consisted of the opposite sectors from the same tomatoes. In sampling canned tomatoes, an entire pint can from the different lots was blended in the Waring Blendor and samples taken from this.

In sampling *snap beans*, the solids were drained from the liquid on a flat sieve having a mesh of nine per inch, and for a specified time. Analyses were made separately on the solids and the liquid.

In sampling *peaches and pears*, the solids were drained from the liquid in a manner similar to that used with the beans. An homogeneous slurry was made for uniform sampling of the solids with the Waring Blendor, and according to recommendations of the Association of Vitamin Chemists (1947). Solids and liquid were analyzed separately.

Canned Snap Beans and Losses of Ascorbic Acid With Cutting and Blanching: The amount of ascorbic acid in canned snap beans, with several different cuts of pods and blanching versus no blanching of the pods, was determined. In the first series of tests, the pods were cut into pieces of three different sizes and blanched in an open kettle before packing and processing. In the second series, pods were cut into similar pieces but were packed raw into cans, exhausted of air in the can, and processed five minutes longer than the blanched beans. Results of the analyses for ascorbic acid on these lots (given Table 1) show that with much cut surface of the pod there were striking losses of vitamin C.

In all tests, analyses were made separately of ascorbic acid in the liquid and solids in the can. The ascorbic acid in both was used in calculating total amount lost with canning and storage.

In Series 1, actual destruction of vitamin C ranked in the following order: 44.4 per cent with the inch cuts; 60.1 with the one-fourth-inch; and 71.4 with the "French" cuts. "French cut" is the term applied to thin, narrow, lengthwise strips of the pods and may be done by mechanical devices. This cut is also popular for frozen snap beans.

TABLE 1

Ascorbic Acid in Canned Snap Beans With Different Cuts of Pods and Two Types of Packs

Method of canning	Type of cut	Ascorbic acid in			Retention raw pack vs. precook		Total destruction in entire contents of can
		Raw beans	Canned snap beans		In solids	In canning liquid	
			Solids	Liquid			
		mg./ 100 gm.	*mg./ 100 gm.*	*mg./ 100 ml.*	*pct.*	*pct.*	*pct.*
Raw beans after harvest	Whole pod	25.89	.....	.....	.....	.....	.....
Raw pack. Exhausted in can. Processed 30 min.	1-inch	.....	10.89	10.2	42.1	39.4	18.5
Precooked 5 min. Packed hot in can. Processed 25 min.	1-inch	.....	6.8	7.6	26.3	29.4	44.3
Raw pack. Exhausted in can. Processed 30 min.	¼-inch	.....	11.8	6.7	45.6	25.9	28.5
Precooked 5 min. Packed hot in can. Processed 25 min.	¼-inch	.....	5.0	5.3	19.4	20.5	60.1
Raw packs. Exhausted in can. Processed 30 min.	French cut—lengthwise in thin slices	.....	8.9	7.2	34.4	27.9	37.7
Precooked 5 min. Packed hot in can. Processed 25 min.	French cut—lengthwise in thin slices	.....	3.5	3.7	13.9	14.7	71.4

In Series 2, where the different cuts were packed raw into the cans, losses of ascorbic acid were considerably less than with blanching, and averaged 18.5 per cent for the inch cuts; 28.5 for the one-fourth-inch; and 37.7 for the "French." For both series the data show the "French" cut was the most wasteful of vitamin C and that the loss became considerably greater with blanching, *viz.*, 37.7 per cent unblanched as compared with 71.4 blanched. The same comparative difference was evident with the other two cuts, but was not as great.

The objective in canning is to produce a high quality and safe product and to retain as much of the original nutrients as possible. According to the scores of judges, the raw-pack beans outclassed the blanched ones in texture and flavor. With this method the texture was rated much more desirable in such succulent beans as the Commodore or Kentucky Wonder varieties. In these varieties there was a tendency for softening or "mush-

ing" with precooking and processing for the 25 minutes considered essential for a safe product. Time for processing was that recommended by the U. S. Department of Agriculture Bureau of Home Economics (1944). Altitude increases in pressure are necessary to secure a temperature of 116°C.(240°F.) in the pressure cooker. The pressure cooker used in these tests was equipped with a Taylor thermometer, as well as a gauge.

It is probable that with stronger-flavored beans blanching would be preferred, to produce a mild-flavored canned product.

Distribution of Vitamin C in Solids and Canning Liquid: Data (Table 1) show the solid portion of the raw-pack beans contained more ascorbic acid than the liquid; whereas, with blanched beans the distribution of ascorbic acid between solids and liquid tended toward equalization.

Vitamin C losses could be greater than those recorded in this experiment if the vegetable was not handled speedily during the harvest, preparation, and processing, as was the case here.

Heat Penetration Good by Both Methods: The raw-pack technique as described for these tests proved safe—with no spoilage of the product. Small maximum thermometers, such as used by canneries, were imbedded in the center of the cans of beans packed by the two methods described. The thermometers recorded 116°C.(240°F.) within similar processing times.

Preventing High Blanching Losses of Ascorbic Acid: Considerable amounts of water-soluble vitamin C dissolve out in the blanching water. Some of this may be conserved by covering the packed cans of beans with it. With more cut surface of the pods, a greater amount of ascorbic acid leaches out into the blanching water. In the "French" cuts, a large amount of the initial ascorbic acid dissolved out into the blanching water, and with such cuts a considerable saving of vitamin C was effected when the blanching water was used to cover the beans. In one lot of the "French-cut" pods such differences were 24.8 per cent retained in the solids with blanching water used in the can, as compared with 3.4 with fresh brine. If blanching water is not used in the can, it may be re-used several times as a blanching bath with such mild-flavored vegetables as snap beans. This builds up a higher concentration of ascorbic acid in the bath and prevents such high losses from the solids.

If the liquid in the can contains more ascorbic acid than the solids, then it is taken up by the solids until there is an approximate equalization of ascorbic acid between solids and liquid.

Enforcement With Ascorbic Acid: It is possible, by enforcing the can of beans with small amounts of l-ascorbic acid or by adding it to the blanching water, to build up greater values of vitamin C in the solid portion, which is the part usually consumed.

Two hundred milligrams of l-ascorbic acid per pint jar were added to different lots of canned snap beans. Retention after processing and storage was 83.3 per cent. Vitamin C was almost equally divided between the solid portion and the liquid, as in the unenforced blanched lots. Texture was somewhat firmer with the added ascorbic acid and in some varieties of snap beans there was less tendency for the cuticle to slough. Addition of the acid had no effect in preventing fading of the green color of

the beans. A slightly perceptible flavor was noted with addition of 200 mg., but was not undesirable.

Canned Tomatoes and Effect of Cutting and Precooking Upon Ascorbic Acid Values: Techniques in this series of tests with canning tomatoes were similar to those used with beans. In Series 1 tomatoes were packed raw into cans, exhausted in the can, and processed in a hot-water bath. In Series 2 they were preheated in an open kettle before packing and processing.

The small hybrid tomatoes used in these tests were about one and one-half to two inches in diameter. These fresh, raw tomatoes contained 33.1 mg./100 gm. ascorbic acid. The Bounty tomato, with which this hybrid was crossed, averaged 25.1 mg./100 gm. ascorbic acid. As previously mentioned, vines were pole-supported, in order to expose the fruit to more sunshine, to hasten ripening; this may also have increased the ascorbic-acid values.

TABLE 2

Ascorbic Acid in Home-Canned Tomatoes[1]

Type of pack	Methods	Moisture	Ascorbic acid moist basis	Ascorbic acid retained	Total destruction
		pct.	*mg./100 gm.*	*pct.*	*pct.*
Raw, fresh	.....	94.1	33.1	.....	.....
Whole tomatoes. Raw-pack	Pack by pressing to fill spaces. Process 50 min.	95.0	23.26	71.3	29.7
Quartered tomatoes. Raw-pack	As above	94.3	23.49	71.0	29.0
Whole tomatoes. Preheat	Hot pack. Process in can 20 min.	94.3	20.75	63.0	37.3
Quartered tomatoes. Preheat	Hot pack. Process in can 20 min.	94.4	19.12	58.0	42.2
Tomatoes simmered and sieved	Filled hot into can. Processed 15 min. in can.	94.2	19.00	60.0	40.0

[1] The variety canned was a hybrid designated "164-11 × Bounty."

Vitamin C retention was good with canning and storage. Data (Table 2) show that retention was 71 per cent of the initial ascorbic acid for either the whole or the quartered raw-packed tomatoes. A more economical pack is effected if large tomatoes are cut into quarters.

In Series 2, where the whole tomatoes had undergone preheating for three minutes and the quartered tomatoes for two minutes, vitamin C retention averaged 63 and 58 per cent, respectively, with losses eight to 13 per cent more than in those packed raw into cans. Loss of ascorbic acid was approximately the same for the sieved tomatoes.

The judges rated the texture and flavor of the raw-packed tomatoes better than those preheated before processing.

Canned Peaches and Pears, With or Without Enforcing With l-Ascorbic Acid: Two series of tests were made in canning pears and peaches, and the ascorbic acid in the canned fruit evaluated. In Series 1 the fruit

was packed raw into cans and processed in a water bath as described under "Experimental Procedure." In Series 2 a similar canning procedure was used, but the fruit was enforced with powdered l-ascorbic acid. Experimental data have shown that l-ascorbic acid is identical in chemical structure and biological value with naturally-found ascorbic acid.

The fresh peaches and pears used for canning were purchased at local markets. Fresh peaches contained 8.5 to nine mg./100 gm. and pears four to six mg./100 gm. ascorbic acid. The raw-packed, canned peaches contained 3.9 mg./100 gm. in the solids and 3.72 in the juice; whereas, the pears contained 1.06 mg./100 gm. in the solid portion and 1.21 in the juice.

TABLE 3

Ascorbic Acid in Home-Canned Peaches and Pears, Fortified and Unfortified With Ascorbic Acid

Fruit	Added l-ascorbic acid	Part tested	Weights in pint can	Ascorbic acid	Ascorbic acid in pint jar	Ascorbic acid retained from l-ascorbic acid		Total retention in entire content of jar
	mg.		*gm.*	*mg./100 gm.*	*mg.*	*mg.*	*pct.*	*pct.*
Elberta peaches, halved	None	Solids	293.70	3.92	11.51	.....	.....	.....
		Juice	192.50	3.72	7.16	.....	.....	.....
	200	Solids	299.60	35.45	106.21	94.69	47.34	78.62
		Juice	185.75	37.55	69.75	62.56	31.28	.....
	550	Solids	291.60	96.85	282.41	270.98	49.22	80.49
		Juice	187.45	95.50	179.00	172.00	31.27	.....
Bartlett pears, quartered	None	Solids	285.55	1.06	3.03	.....	.....	.....
		Juice	214.80	1.21	2.60	.....	.....	.....
	200	Solids	257.20	22.78	58.59	55.56	27.78	58.00
		Juice	239.95	26.24	62.96	60.36	30.18	.....
	554	Solids	308.50	65.00	200.52	197.49	35.64	59.74
		Juice	211.40	64.39	136.12	133.52	24.10	.....

In the enforced canned fruit (as indicated Table 3), the powdered l-ascorbic acid was used in amounts ranging from 200 to 500 mg. per pint. With addition of 200 mg., the peaches retained 78.62 per cent of the added ascorbic acid and the pears 58 per cent. Retention was determined by subtracting the amount of ascorbic acid in the pint jar of unfortified fruit from that in the fortified fruit.

Lower retention of ascorbic acid in the pears was no doubt due in part to the longer processing period (15 minutes) in the water bath, which was necessary to soften and preserve this low-acid fruit at the 7,200-foot elevation. Slight irregularities in retention with different amounts of ascorbic acid used per pint of the same fruit are evident in the tabular data (Table 4). Such small differences were probably due to variables in processing, such as differences in head-space or the amount of air locked in the fruit.

The enforced canned fruit showed a striking improvement in both flavor and color with addition of 200 mg. powdered l-ascorbic acid to a

pint jar. No undesirable flavors developed with amounts up to 500 mg. With the larger amount both color and flavor were good after 20 months in storage. Peaches held their natural color with addition of 125 mg.

The unfortified home-canned pears darkened in color, and off-flavors developed with storage. They were almost devoid of vitamin C. Browning or darkening occurred oftener at the top of the jar, although frequently all pieces in the jar darkened.

Although unfortified home-canned peaches held their color better than pears, they, too, were improved with addition of l-ascorbic acid. It was added to the syrup just before pouring it over the fruit and processing it in the can. In both fruits the added ascorbic acid came to an approximate equilibrium between the solid portion and the juice after processing and storage. In canned fruit both fruit and juice are consumed, and there is no nutritional waste of this vitamin as may occur with the liquid of the canned vegetables, if discarded. In such instances approximately one-half the vitamin content of snap beans may be wasted.

The high quality of the fortified canned fruit shows that the synthetic ascorbic acid is a valuable adjunct in the home-canning of these fruits. Cost is not prohibitive for such use—about 0.6 cent for 200 mg. purchased in lots of 125 gm. (U.S.P. Grade, Merck; most druggists are willing to stock this grade for such purpose).

SUMMARY AND CONCLUSIONS

Nutritive values of home-canned vegetables and fruit may be lessened materially by procedures in canning.

The extent of the vitamin C loss in home-canned snap beans differs according to methods of preparation before canning. Blanching and scalding in hot water had the most detrimental effect on loss of the water-soluble vitamin C in snap beans. Greater amounts of ascorbic acid leached out with more cut surface of the tissue. In the lots of snap beans blanched before processing, losses ran as high as 71.4 per cent with "French" cuts, in which pods were shredded lengthwise. Losses were 60.1 per cent with one-fourth-inch crosscuts of the pods, and 44.4 per cent with inch cuts.

In the blanching procedure, utilization of blanching water to cover the beans in the can conserved more ascorbic acid than if fresh water was added, and to a greater degree where more had leached out, as occurred with the "French" cut.

With a high concentration of ascorbic acid in the blanching water, losses became correspondingly less in the tissue. A more concentrated blanching bath can be built up by re-using the same liquid for blanching several lots of the vegetable, or by adding some l-ascorbic acid to it.

If the cut pods were packed raw into cans and processed in the can, losses were reduced to 37.7 per cent for "French" cuts, 28.5 for the one-fourth-inch cuts, and 18.5 for the inch cuts. There was no spoilage of the raw-pack beans, and quality ratings were high with the mild-flavored varieties tested.

Tomatoes contained more initial ascorbic acid than other commodities tested in this study. Losses with canning and storage were about 29 per cent if raw-packed, and 37 to 42 per cent if blanched in an open kettle.

Home-canned pears were practically devoid of vitamin C. Enforcing this canned fruit with l-ascorbic acid had striking effects upon improving quality, by preventing darkening or browning of the fruit and by preserving natural flavors. Approximately 58 to 59 per cent of the l-ascorbic acid added to canned pears was retained with processing and storage for six months.

Enforcing home-canned peaches with l-ascorbic acid had similar desirable effects. Retention after canning and storage was higher with peaches than with pears, probably because of a shorter processing time (15 minutes less). Approximately 78 to 80 per cent of that added was retained in home-canned peaches with storage for six months.

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PHYSIOLOGICAL STUDIES OF THE SEEDS OF GRAPEFRUIT (*CITRUS PARADISI*, MACFAD.)

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During the past 15 or 20 years in the United States increasing quantities of grapefruit (*Citrus paradisi*, MacFad.) have been processed and distributed as canned juice or as fruit segments. This change in method of marketing these fruits has created new problems, an important one of which is the disposal of cannery waste. Very early in the history of the industry a use was found for the peel and expressed pulp of citrus fruits by dehydrating and converting them into cattle feed. Somewhat later, additional use of the waste was made by extracting essential oils and pectin from the peel, and fats and other substances from the seeds.

It occurred to the writers that citrus seeds might be utilized as a source of still other compounds, such as vitamins and that, furthermore, the quantity of these substances might be increased by sprouting the seeds.

Although sprouted seeds have long been used as an article of diet in certain parts of the world, it has been only in relatively recent years that very much attention has been devoted to the effect of germination upon nutritive value. Among the earlier workers in this field were Chick and Delf (1919), who reported that germinated peas and lentils showed a higher anti-scorbutic value than did the dry seeds and, a little later, Santos (1922) stated that the vitamin B content of mung beans was enhanced by sprouting. Other tests with various legumes have indicated that germination results in an increase in vitamin C, Rose and Phipard (1937), or in vitamin B, Cheldelin and Lane (1943), or in both of these vitamins or vitamin groups, Burkholder and McVeigh (1945). French, Berryman, Goorley, Harper, Harkness, and Thacker (1944) found that, generally speaking, sprouted legume seeds are an excellent source of ascorbic acid, fair for riboflavin, and poor for thiamine.

Wai, Bishop, Mack, and Cotton (1947) found that germinated soybeans showed a higher content of carotene, riboflavin, niacin, and ascorbic acid than did the ungerminated seeds.

Seeds of certain cereals have also been shown to increase in nutritive value as the result of germination. Bogart and Hughes (1935) reported that sprouting of oats increased the ascorbic acid content and results of Burkholder (1943) indicated that corn, wheat, oats, and barley all show increases in vitamin B during germination.

MATERIAL AND METHODS

These investigations were begun in January, 1948, and continued for a period of 18 months. Three separate experiments were performed. Seeds employed in these studies were removed from fresh grapefruit which had

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been shipped from Florida. Fruits employed in the first two experiments were shipped from Lake Alfred and those in the third from Winter Garden. As soon as the grapefruit arrived in Pittsburgh they were stored at 6°C.(43°F.) and held there until they could be sampled. In each experiment the seeds were divided into two lots, one of which was prepared for germination and the other analyzed immediately.

Seeds were germinated by the following method: They were first soaked for an hour in a suspension of "Arasan," for the purpose of sterilizing the seed coats. They were next placed in moist chambers containing about an inch of moist sand and held at 30°C.(86°F.).

The ungerminated seeds were rinsed in water and scrubbed with a brush to remove the mucilaginous exudate which adhered to the coats. Excess moisture was removed with paper towels and the seeds allowed to dry for an hour at room temperature.

Fifty seeds were selected for each sample in each analysis and these replicated five times. Fresh weights were obtained of the whole seeds, as well as of the embryos, after seed coat and testae had been removed. Likewise, the dry weight was obtained for the whole seeds and embryos only of comparable lots of seeds.

Ascorbic Acid: Embryos of the ungerminated seeds were ground with sand in a mortar and extracted with eight per cent metaphosphoric acid. The extract was centrifuged and the supernatant liquid titrated with standardized 2,6-dichlorophenolindophenol.

Total carotene was determined by the method of Miller, Winston, and Schomer (1940). This consisted of extracting the embryos with acetone and transferring the extract to petroleum ether. Saponification with methyl alcoholic potash, followed by washing with water, removed chlorophylls, if present, as well as the anthoxanthins. The xanthophylls were removed by washing the petroleum ether extract with 92 per cent methanol. The extract of carotenes was filtered through anhydrous sodium sulfate and then compared with a standard solution of beta-carotene, using a Klett-Summerson colorimeter.

The germinated seeds were allowed to grow until the root radicals were between one and two inches long. No seeds were used if the epicotyls had attained a length greater than one inch. Seeds were counted out into replicates of 50. Seed coats and testae were removed and discarded. Fresh weights of the embryos were obtained before sampling and dry weights obtained with comparable lots of embryos of germinated seeds.

Methods of analyses of the germinated seeds were identical with those used for the ungerminated ones.

RESULTS

The amount of ascorbic acid in the embryos of ungerminated and germinated seeds is presented (Table 1). On a fresh weight basis the ungerminated seeds showed a range of 1.24 to 2.62 mg. and the germinated seeds a range of 13.57 to 26.38 mg. per 100 gm. Averages for each of the three experiments were 1.492, 1.336, and 2.242 mg., respectively, per 100 gm. ungerminated seeds; and 15.254, 20.590, and 16.206 mg., respectively, per 100 gm. germinated seeds. An overall average showed 1.69 mg. of ascorbic

acid per 100 gm. ungerminated embryos and 17.346 mg. per 100 gm. germinated embryos.

Since germinating seeds absorb large quantities of water, a more accurate appraisal of changes occurring during germination may be obtained by expressing results on a dry-weight basis. Expressed in this manner the ascorbic acid in the ungerminated embryos ranged from 2.55 to 5.29 mg., and in the germinated, from 36.41 to 56.63 mg. per 100 gm. The overall average for ascorbic acid on the dry weight basis was 3.358 mg. per 100 gm. of ungerminated embryos, and 44.791 mg. per 100 gm. of germinated embryos.

TABLE 1

Ascorbic Acid Content of Ungerminated and Germinated Embryos of Grapefruit Seeds

Sample	Ascorbic Acid [1]			
	Ungerminated seeds		Germinated seeds	
	Fresh weight	Dry weight	Fresh weight	Dry weight
Experiment I				
1	1.45	2.87	13.57	36.41
2	1.39	2.73	15.17	40.72
3	1.82	2.86	14.50	38.89
4	1.40	2.77	15.66	42.02
5	1.40	2.76	17.37	37.02
Average	1.492	2.792	15.254	39.012
Experiment II				
1	1.24	2.55	26.38	56.63
2	1.40	2.88	19.19	51.87
3	1.44	2.95	19.22	51.94
4	1.31	2.68	18.34	49.56
5	1.29	2.64	19.82	53.58
Average	1.336	2.740	20.590	52.716
Experiment III				
1	1.64	3.32	14.45	36.66
2	2.55	5.17	15.58	41.31
3	1.82	3.69	17.78	47.16
4	2.62	5.29	18.75	49.72
5	2.58	5.22	14.47	38.38
Average	2.242	4.538	16.206	42.646
Grand Average	1.690	3.358	17.346	44.791

[1] Mg. per 100 gm.

From the above results it will be observed that as a result of germination of grapefruit seeds there occurred an 11-fold increase in ascorbic acid, based on fresh weight, and a 13-fold increase expressed on a dry-weight basis.

Total carotene in grapefruit seeds also increased as result of germination. These results appear (Table 2). On a fresh-weight basis carotene in the ungerminated seeds ranged from 0.00 to 0.09 mg. and in the germinated seeds the range was between 0.13 and 0.20 mg. per 100 gm.

Averages for each of the three experiments were 0.026, 0.036, and 0.018 mg. in the ungerminated seeds, and 0.164, 0.162, and 0.180 mg. in the germinated. Averaging all replicates in all experiments the ungerminated seeds were found to contain 0.027 mg. of carotene per 100 gm. The overall average for germinated seeds was 0.168 mg. per 100 gm. of fresh weight.

Overall average for carotene on a dry-weight basis was as follows: ungerminated seeds, 0.047 mg. per 100 gm.; germinated seeds, 0.462 mg. per 100 gm.

TABLE 2

Total Carotene Content of Ungerminated and Germinated Embryos of Grapefruit Seeds

Sample	Total Carotene[1]			
	Ungerminated seeds		Germinated seeds	
	Fresh weight	Dry weight	Fresh weight	Dry weight
Experiment I				
1	0.02	0.03	0.16	0.48
2	0.01	0.02	0.13	0.37
3	0.04	0.08	0.19	0.57
4	0.04	0.08	0.16	0.43
5	0.02	0.04	0.18	0.51
Average	0.026	0.050	0.164	0.472
Experiment II				
1	0.00	0.00	0.16	0.45
2	0.05	0.08	0.15	0.41
3	0.03	0.05	0.18	0.49
4	0.09	0.14	0.16	0.45
5	0.01	0.02	0.16	0.44
Average	0.036	0.058	0.162	0.448
Experiment III				
1	0.02	0.03	0.13	0.35
2	0.02	0.04	0.18	0.42
3	0.01	0.03	0.19	0.51
4	0.02	0.04	0.20	0.52
5	0.02	0.03	0.20	0.54
Average	0.018	0.034	0.180	0.468
Grand Average	0.027	0.047	0.168	0.462

[1] Mg. per 100 gm.

Increase in carotene content as result of germination was six-fold on a fresh weight basis and 10-fold when expressed on a dry-weight basis.

ASCORBIC ACID AND CAROTENE CONTENT OF WHOLE SEEDS

It will be noted that, thus far, all results have been expressed in terms of the embryos only, but results are expressed in terms of the whole seeds (Table 3). This has been done to permit estimation of the yield of ascorbic acid or carotene that might be expected from the seeds as they are discharged from the cannery.

As will be seen from the table, 100 gm. of whole seeds contained 0.936 mg. ascorbic acid and the same quantity of germinated seeds contained

10.693 mg. On a dry-weight basis the ungerminated seeds contained 2.073 mg. and the germinated seeds 30.956 mg. ascorbic acid per 100 gm.

Carotene content of the fresh whole seeds was found to be 0.013 mg. in the ungerminated, and 0.106 mg. per 100 gm. in the germinated seeds. On a dry-weight basis the carotene content averaged 0.020 mg. for the ungerminated and 0.273 mg. per 100 gm. for germinated seeds.

TABLE 3

Ascorbic Acid and Total Carotene Content of Ungerminated and Germinated Grapefruit Seeds[1]

Ascorbic Acid [2] (Mg. per 100 gm.)

Experiment	Ungerminated seeds		Germinated seeds	
	Fresh weight	Dry weight	Fresh weight	Dry weight
I	0.86	1.69	9.59	32.03
II	0.81	1.10	12.38	35.50
III	1.14	3.43	10.11	25.34
Average	0.936	2.073	10.693	30.956

Total Carotene [2] (Mg. per 100 gm.)

Experiment	Ungerminated seeds		Germinated seeds	
	Fresh weight	Dry weight	Fresh weight	Dry weight
I	0.01	0.02	0.11	0.28
II	0.02	0.02	0.10	0.25
III	0.01	0.02	0.11	0.29
Average	0.013	0.020	0.106	0.273

[1] Values based on weight of whole seed; i.e., embryos plus seed coat. [2] Averages of five replicates.

Using the values (presented Table 3) it can now be estimated that a ton of grapefruit seeds on the average would contain 9.33 gm. ascorbic acid and 0.263 gm. carotene. If a ton of seeds were germinated the ascorbic acid content would be increased to 177.6 gm. and the carotene to 1.59 gm. Assuming 90 per cent of the total carotene to be beta-carotene, as is usually the case with plant products, a ton of grapefruit seeds, when germinated, would contain approximately 2,386,325 I. U. of vitamin A.

DISCUSSION

Results of these experiments do not indicate that grapefruit seeds, whether germinated or ungerminated, constitute an especially rich source of either ascorbic acid or carotene. However, they serve to indicate to what extent the nutritive value of cattle feed from cannery waste may be enhanced by addition of seeds to the waste peel and pulp; and also to emphasize the possible advantage of germinating the seeds prior to combining them with the waste. There is always the possibility, too, of utilizing such by-products for supplementing other types of commercial feeds. The practicability, in any case, would depend upon cost of utilizing the seeds as compared to the price of synthetic ascorbic acid and that of commercial supplies of carotene now available.

A certain amount of fats, carbohydrates, and proteins are contained in the seeds and the food value of these substances should also be included in determining the practicability of utilizing citrus seeds in this manner.

The various questions raised will not be completely answered until tests are made with experimental animals and until assays have been made for the vitamins included in the so-called "B Complexes." Especial attention should be devoted to a study of vitamin P, or rutin, which is known to be present in citrus fruits and which has been shown to be of use in strengthening capillary walls in animals.

Citrus seeds are of particular interest to the physiologist because the cotyledons frequently develop chlorophyll in the absence of light, a characteristic possessed by very few species of angiosperms. Toward the end of the season it is a common occurrence to find in grapefruit not only seeds with green cotyledons but seeds which have begun to sprout while still within the fruit. It should be interesting to study physiological changes in the grapefruit seeds during the entire commercial season, to determine if there is a seasonal increase in vitamin content of ungerminated seeds.

The preference for seedy grapefruit, shown by canners, accounts for the large quantities of seeds available at the canneries. The officials of one cannery alone have stated that eight or 10 tons of seeds per week are normally recovered during the height of the canning season.

SUMMARY

Observations were made on the ascorbic acid and total carotene contents of ungerminated and germinated seeds of the Duncan grapefruit (*Citrus paradisi*, MacFad.).

Averaging all the results the ungerminated embryos were found to contain 1.690 mg. and the germinated embryos 17.346 mg. ascorbic acid per 100 gm. of fresh material. Based on the same quantity of dry weight, the values were 3.358 mg. in the ungerminated seeds and 44.791 in the germinated.

Total carotene of the embryos averaged 0.027 mg. per 100 gm. in the ungerminated and 0.168 mg. per 100 gm. in the germinated seeds, expressed on fresh weight basis. Computed on the dry weight basis ungerminated seeds contained 0.047 mg. and germinated seeds 0.462 mg. of carotene per 100 gm.

Increase in ascorbic acid content of the embryos as a result of germination was 13-fold and for the carotene 10-fold, based on dry weights.

Expressed in terms of the dry weight of the whole seed (embryo plus seed coat) average values were as follows: ascorbic acid, ungerminated—2.073 mg. per 100 gm.; germinated—30.956 mg. per 100 gm. Carotene contents of the whole seeds, based on dry weight were as follows: ungerminated—0.020 mg. per 100 gm.; germinated—0.273 mg. per 100 gm.

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EFFECT OF ACIDS, SALT, SUGAR, AND OTHER FOOD INGREDIENTS ON THERMAL RESISTANCE OF *BACILLUS THERMOACIDURANS*[1]

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In the course of an investigation on the effect of various ingredients of acid food products on the thermal resistance of microorganisms, observations were made on the influence of such substances as acids, salt, sugars, certain spices, sodium benzoate, and ascorbic acid on the thermal resistance of *Bacillus thermoacidurans* in tomato juice. This organism was originally described by Berry (1933) and was implicated as a cause of spoilage in canned tomato juice. Since then considerable work has been reported on spoilage of tomato juice by this organism and the process requirements for its destruction in this product.

EXPERIMENTAL PROCEDURE

Source of Microorganism: Strain number 8038 of *B. thermoacidurans* used in this study was obtained from the American Type Culture Collection which listed the culture as *Bacillus coagulans* in accordance with Bergey's Manual [Breed, Murray, and Hitchens (1948)].

Preparation of Substrate: The tomato juice used in the following experiments was prepared from a single lot of sound fresh tomatoes which had been carefully washed, trimmed, and put through a cyclone finisher. The juice was then filled into No. 10 (603 x 700) cans, sealed, and stored at a temperature of −23°C.(−10°F.). As needed, a can of the frozen juice was removed from storage, thawed as rapidly as possible, filtered through a screen to remove any lumps of coagulated material, filled into eight-ounce, screw-cap medicine bottles, sterilized for 15 minutes at 121.1° C.(250°F.), cooled, and stored in a refrigerator at 0°C.(32°F.).

The pH of the freshly thawed juice, as well as that of the sterilized juice, was between 4.4 and 4.5.

Composition and Preparation of Medium: In the preparation of the medium used for growing the spore crop as well as for recovery counts, techniques substantially the same as those proposed by Stern, Hegarty, and Williams (1942) were employed. Composition of the special proteose peptone acid agar (PPAA) medium (Table 1) that was employed by these authors was modified only to the extent that 0.1 per cent soluble starch was added to each batch of medium. Incorporation of the starch resulted in: 1) counts averaging 30 per cent higher than the same dilutions plated out on the medium without the starch; and 2) making it possible to treat the plates after incubation with a dilute solution of iodine as recommended by Scott (1949). The resultant starch-iodine reaction caused both surface

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and subsurface colonies of *B. thermoacidurans* to stand out prominently against the blue agar background, thereby enabling more rapid and accurate counts to be made.

Method of preparing the PPAA medium was also altered somewhat from that proposed by Stern *et al.* (1942). In the present study, the nutrient solution (Solution A), containing an additional 0.1 per cent soluble starch, was prepared and adjusted to pH 5.0 with one normal hydrochloric acid. After Solution B was made up, the two solutions were heated for only four minutes at 121.1°C.(250°F.), rather than sterilized separately for 30 minutes at this temperature, as originally recommended. This procedure dissolved the agar, and when Solutions A and B were combined a homogeneous suspension was obtained which was easily dispensed into test tubes. These tubes were then sterilized for 15 minutes at 121.1°C. (250°F.).

TABLE 1

Composition of Proteose Peptone Acid Agar Medium (Stern, Hegarty, and Williams, 1942)

Solution A:	
Proteose peptone (Difco)	5 gm.
Yeast extract (Difco)	5 gm.
Glucose	5 gm.
K_2HPO_4	4 gm.
Distilled water	500 ml.
Solution B:	
Agar	20 gm.
Distilled water	500 ml.

Plugging of the filled test tubes was greatly facilitated by use of glass vials in place of the conventional cotton plugs, as suggested by Wicks (1948). These vials, approximately 70 mm. long with an outside diameter two to three mm. greater than that of the test tube, contained cotton to a depth of about one inch. Use of such vials not only saved considerable time in tubing of the medium but they could be used repeatedly without need of replacing the cotton.

Preparation of Spore Inoculum of B. thermoacidurans: Techniques employed in preparation of the spore inoculum were in accordance with those outlined by Stern, Hegarty, and Williams (1942). It was found that 200 ml. of saline suspension, representing the combined growth from four eight-ounce, screw-cap medicine bottles, contained approximately 25 million spores of *B. thermoacidurans* per ml.

Throughout this study, sufficient inoculum was added to a known volume of tomato juice, with or without added food ingredients, so as to give a final concentration of 100,000 spores of *B. thermoacidurans* per ml. of substrate.

Method for Determining Thermal Resistance: A modification of the multiple tube method of Esty and Williams (1924) was followed in obtaining rate of destruction data. In preparation of the thermal death time (TDT) tubes, 120-cm. lengths of Pyrex glass tubing, with an outside diameter of nine mm. and a wall thickness of one mm., were cut into 30-cm.

sections. These sections were soaked in dichromate-sulfuric acid cleaning solution overnight and then rinsed thoroughly with distilled water, after which they were sealed at their center by means of an oxygen flame. The resulting TDT tubes, approximately 15 cm. long, were then plugged with cotton and sterilized by heating in an electric oven for three hours at 204.4°C.(400°F.).

Two-ml. aliquots of the substrate, containing the standard inoculum of 100,000 spores of *B. thermoacidurans* per ml., were filled into the sterilized TDT tubes by means of sterile 10-ml. serological pipettes; the tubes were replugged and sealed by means of an oxygen flame.

Duplicate or triplicate TDT tubes were employed to obtain rate of destruction data for each time interval at a given temperature. The TDT tubes for each separate time interval were placed in a wire mesh basket, which facilitated transfer of the tubes into the oil bath and from the oil bath into the ice water.

The oil bath, containing approximately five gallons of oil, was well insulated and maintained temperatures within ±0.12°C.(±0.2°F.) by means of a Beckman mercury to wire thermoregulator, an immersion heating unit of 500-watt capacity, a Precision Scientific Company "Normally Closed" relay box, and an electric stirrer.

After the oil bath was adjusted to the desired trial temperature, the wire baskets containing the TDT tubes were immersed in the oil bath for various experimental heating times. At the expiration of these times, the baskets were removed from the oil bath, transferred rapidly to an ice-water bath, and agitated violently to assure rapid cooling of the tube contents.

All TDT tubes were subcultured in the following manner upon expiration of the heating period: They were first freed of oil by wiping on clean toweling, one end was flamed thoroughly and then opened by means of an apparatus similar to that described by Williams (1929). Inasmuch as the standard pipettes would not fit into the TDT tube, it was necessary to transfer the contents of each TDT tube to a sterile test tube. From the test tubes one-ml. aliquots were transferred by means of sterile pipettes to appropriate dilution bottles from which duplicate plates of PPAA medium were made for each TDT tube. After the agar had solidified, the plates were put in an incubator maintained at 37°C.(98.6°F.) and held for at least 72 hours before counting. The numbers of surviving organisms were then plotted against time on semi-logarithmic paper, with a lethal value of 99.99 per cent destruction being taken as the endpoint; i.e., a reduction in count from the initial concentration of 100,000 spores per ml. to a final concentration of only 10 spores per ml.

In those studies where the rate of destruction curves are straight lines, it is customary to describe them by means of the time required for the desired endpoint of destruction and the slope of the rate of destruction curve (Z value) expressed in minutes. Because in the present investigation many of the rate of destruction curves for *B. thermoacidurans* were not straight lines, it was impossible to ascribe Z values to them. Typical thermal death rate curves are shown (Fig. 1). Stiebling (1948) obtained

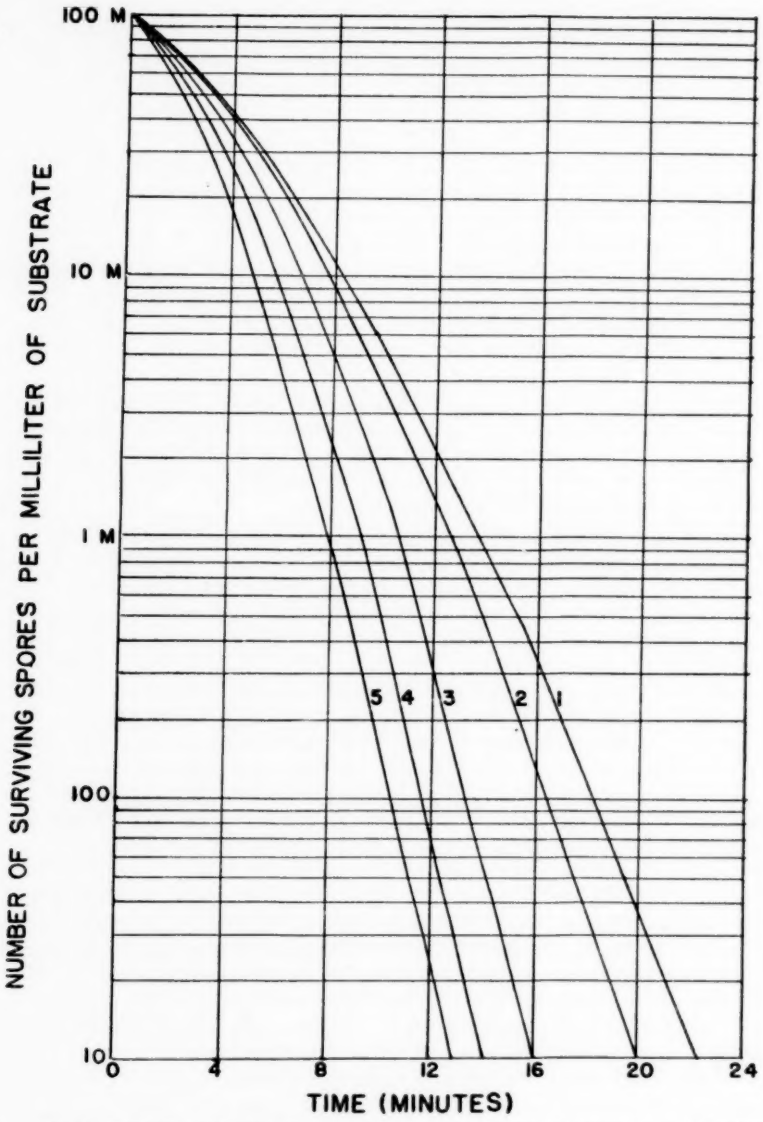

FIG. 1. Thermal death rate curves of *B. thermoacidurans* in tomato juice (100,000 spores/ml.) with and without various concentrations of NaCl.

1. Tomato juice—no salt. 2. Tomato juice with 1 per cent salt. 3. Tomato juice with 2 per cent salt. 4. Tomato juice with 4 per cent salt. 5. Tomato juice with 8 per cent salt.

similar results while working with P.A. 3679, in which the thermal death rates showed a definite lag in the earlier stages of the heating period.

After the time required to effect 99.99 per cent destruction in a given substrate for at least three different temperatures was determined, results were plotted on semi-logarithmic paper with temperature on the abscissa and time on the ordinate. This resulted in the so-called thermal death time curve which in almost every case proved to be a straight line.

Ball (1923) ascribed two characteristics to a TDT curve which serve to describe the thermal resistance of the test organism under the experimental conditions. These were: 1) the z value—which is the slope of the TDT curve expressed as the number of degrees Fahrenheit passed over by the TDT curve in traversing one logarithmic cycle; and 2) the F value—or number of minutes required to destroy an organism at 121.1°C.(250°F.) in any specific medium.

The lag correction factor for the TDT tubes in the constant temperature oil bath was determined by the General Method of Bigelow, Bohart, Richardson, and Ball (1920), a process which integrated the heat penetration data of the TDT tube with the thermal resistance of the test organism. Data obtained with test solutions of tomato juice, 60 per cent sucrose and distilled water at temperatures of 76.6, 100, and 104.4°C.(170, 212, and 220°F.), indicated that the lag correction factor, or amount of time to be subtracted from total experimental heating time, was two and one-half minutes.

The food ingredients, whose effect on the thermal resistance of *B. thermoacidurans* in tomato juice was studied, included the following: sodium chloride and anhydrous dextrose of C. P. grade; sucrose and lactic, acetic and citric acids of Reagent grade; sodium benzoate, ascorbic acid, and oil of clove of U.S.P. quality; and a commercial brand of ground black pepper (1.2 ml. volatile oil/100 gm. pepper).

Effect of Salt: Influence of salt on the thermal resistance of various microorganisms has commanded the interest of various investigators who have reported both increased lethality and a definite protective effect. For investigations on the effect of salt on organisms other than *B. thermoacidurans*, the works of Weiss (1921a), Esty and Meyer (1922), Esty (1925), Viljoen (1926), Headlee (1931), Wallace and Tanner (1931), Baumgartner (1937), and others may be consulted.

Concentrations of one, two, four, and eight per cent salt in tomato juice were studied. Upon addition of the increasing amounts of salt, there occurred a progressive lowering of the hydrogen ion concentration in the tomato juice. Thus, pH ranges for tomato juice and for tomato juice with one, two, four, and eight per cent salt were, respectively: pH 4.4 to 4.5, 4.3 to 4.4, 4.2 to 4.3, 4.1, and 4.0 to 4.1.

On plotting the endpoints of the thermal death rate determinations at the temperatures of 95.6, 100, and 104.4°C.(204, 212, and 220°F.), it may be seen (Table 2) that the effect of increasing the salt concentrations was reflected in more rapid destruction times. The F and z values for *B. thermoacidurans*, obtained by extrapolation of a TDT curve constructed from the above data (Fig. 2), are also presented (Table 2).

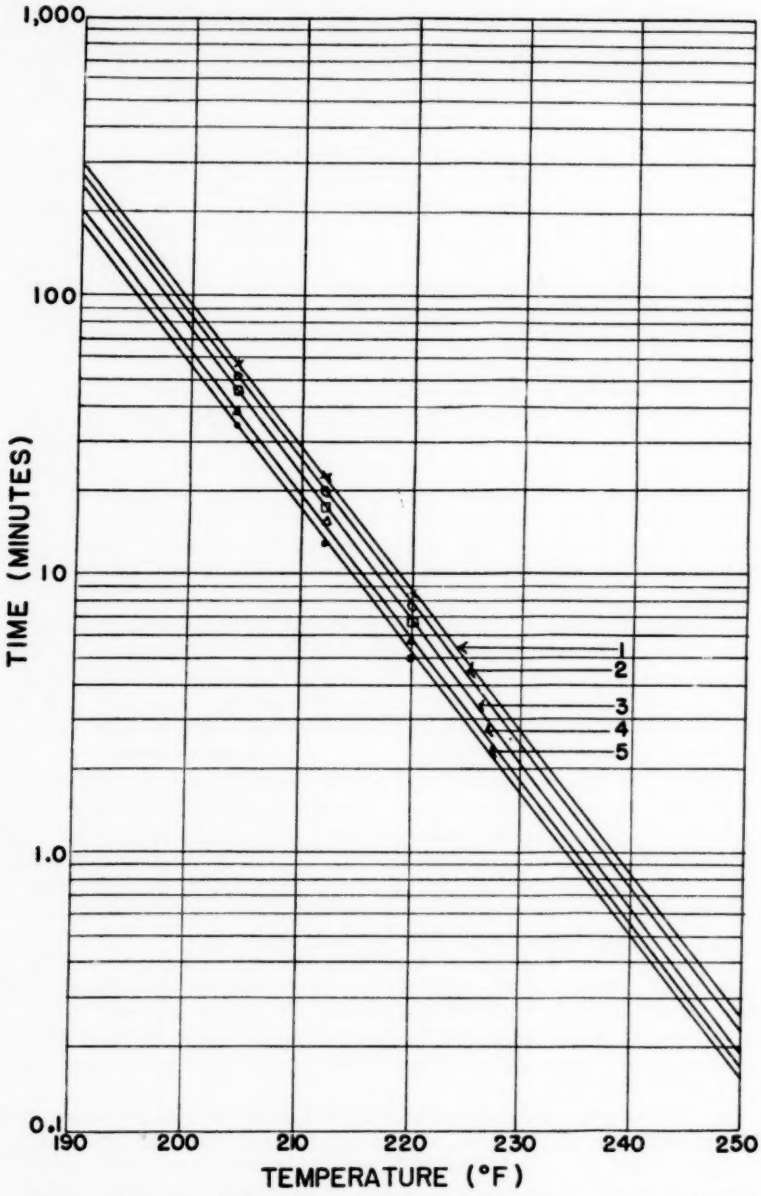

FIG. 2. Thermal death time curves of *B. thermoacidurans* in tomato juice (100,000 spores/ml.) with and without various concentrations of NaCl.

1. Tomato juice—no salt. 2. Tomato juice with 1 per cent salt. 3. Tomato juice with 2 per cent salt. 4. Tomato juice with 4 per cent salt. 5. Tomato juice with 8 per cent salt.

TABLE 2

Thermal Death Characteristics of an Inoculum of 100,000 Spores of B. thermoacidurans per ml. of Tomato Juice Containing Added Food Ingredients

Substrate	pH of substrate	Time in minutes for 99.99% destruction at			F value	z value
		95.6°C. (204°F.)	100°C. (212°F.)	104.4°C. (220°F.)		
Tomato juice	4.4-4.5	55	22	8.2	0.25	19.5
Tomato juice with 1% salt	4.3-4.4	54	19	8	0.23	19.7
Tomato juice with 2% salt	4.2-4.3	45	16	7	0.185	19.2
Tomato juice with 4% salt	4.2	38	14	5.6	0.17	19.4
Tomato juice with 8% salt	4.0-4.1	34	11	5	0.155	18.6
Tomato juice with 10% dextrose	4.3	52	26	9	0.27	20.5
Tomato juice with 25% dextrose	4.3	71	32	11.5	0.32	20.1
Tomato juice with 40% dextrose	4.3	107	38	16	0.43	19.5
Tomato juice with 50% dextrose	4.3	120	45	19	0.52	19.5
Tomato juice with 10% sucrose	4.4	68	23	8.2	0.20	18.2
Tomato juice with 25% sucrose	4.4	78	26	9.5	0.22	19.2
Tomato juice with 40% sucrose	4.4	110	30	11	0.26	18.2
Tomato juice with 50% sucrose	4.4	130	35	12.5	0.28	18.1
Tomato juice with 0.5% lactic acid	3.8-3.9	23	10.5	4.8	0.25	23.6
Tomato juice with 1.0% lactic acid	3.5-3.6	13	6	3.2	0.19	25
Tomato juice with 2.0% lactic acid	3.2-3.3	4.7	2.5	1.6	0.14	30
Tomato juice with 0.5% citric acid	3.8-3.9	23	11	5.6	0.35	25
Tomato juice with 1.0% citric acid	3.5-3.6	14	7.4	4	0.32	27.5
Tomato juice with 2.0% citric acid	3.1-3.2	7	4	2.3	0.29	33
Tomato juice with 0.5% acetic acid	4.0-4.1	30	12	4.5	0.15	20
Tomato juice with 1.0% acetic acid	3.9-4.0	19	8.5	3.4	0.14	21.8
Tomato juice with 2.0% acetic acid	3.7	11	5	2.5	0.125	23.5
Tomato juice at pH 3.8 with acetic acid	3.8	14	6.2	2.3	0.21	22.5
Tomato juice at pH 3.8 with lactic acid	3.8	19	8.6	3.5	0.19	23
Tomato juice at pH 3.8 with citric acid	3.8	21	9.4	4.2	0.14	23
Tomato juice with 0.1% black pepper	4.4-4.5	54	21	8.0	0.23	19.6
Tomato juice with 1.0% black pepper	4.5	43	19	6.5	0.20–	20
Tomato juice with 0.01% oil of clove	4.4-4.5	46	20	7	0.20	19.8
Tomato juice with 0.1% oil of clove	4.4-4.5	40	17	6	0.19	19.6
Tomato juice with 0.1% sodium benzoate	4.4-4.5	50	17	7.4	0.22	19.8
Tomato juice with 200 mg. ascorbic acid/pint of juice	4.4	55	19	8.0	0.24	19.4

Effect of Sugars: It is now generally accepted that addition of sugars or sugar sirups to canned products will enhance the thermal resistance of the microorganisms present in the final container. However, there are reports in the literature that presence of certain sugars decreased the heat resistance of spoilage organisms. Investigations on the general subject of the effect of sugars on thermal resistance of bacteria have been carried out by Weiss (1921b), Peterson, Levine, and Buchanan (1927), Rahn (1928), Williams (1929), Wallace and Tanner (1931), Anzulovic (1932), Fay (1934), Baumgartner and Wallace (1934), Baumgartner (1938), Tanner (1944), and others.

Solutions of 10, 25, 40, and 50 per cent sucrose and dextrose, respectively, in tomato juice were prepared. The sugars were added directly to the sterile juice and final concentration checked by means of an Abbé refractometer.

Results obtained from the various rate of destruction curves of the two sugars, as well as the influence of the different concentrations of sugars on the pH of the substrate are presented (Table 2). It is apparent that an increase in the concentration of either sugar in the tomato juice substrate increased the thermal resistance of *B. thermoacidurans.*

Effect of Acids: That the addition of various organic acids, especially in the case of non-acid foods, resulted in increased efficiency of a given heat treatment has been realized for many years. Feasibility of this practice is evidenced by the acidification of certain products such as artichokes, cauliflower, and onions.

For original references on the effect of acids and pH, the reader is referred to Dickson, Burke, and Ward (1919), Bigelow and Esty (1920), Weiss (1921a and b), Bigelow and Catheart (1921), Esty and Meyer (1922), Williams (1929), Sommer and Sommer (1932), Lang and Dean (1934), Aref and Cruess (1934), Beamer and Tanner (1939), Levine and Fellers (1940), Erickson and Fabian (1942), Skillinglaw and Levine (1943), Baumgartner (1946), Sognefest, Hays, Wheaton, and Benjamin (1948), and others.

Acetic, lactic, and citric acids were added to tomato juice in levels of 0.5, 1.0, and 2.0 per cent. Their effect on the pH of tomato juice is shown (Table 2) as are the times necessary to effect 99.99 per cent destruction of the test inoculum. Increases in the concentration of each acid resulted in increased destruction rates.

Although some investigators have attributed the effect of added acids in reducing the heat resistance of microorganisms to the accompanying change in the hydrogen ion concentration, others, such as Levine and Fellers (1940), have pointed out that toxicity of certain organic acids was due, in part, to the undissociated acid molecules. Therefore, the effect of lactic, acetic, and citric acids in lowering the thermal resistance of *B. thermoacidurans* in tomato juice that had been adjusted to pH 3.8 by means of the above acids was determined. As the pH of the tomato juice substrate was the same with all three acids, variations in destruction times and rates would be attributed to the undissociated acid molecules or the acid radicals.

Destruction times at the various temperatures for the three acids are given (Table 2).

Effect of Spices: It has long been known that certain spices, such as oil of clove, cinnamon, and mustard may exert bacteriostatic or even bactericidal effects against microorganisms at ordinary growth temperatures. However, according to Baumgartner (1946), the toxic effect of various spices and spice oils on microorganisms at processing temperatures did not appear to have ever been investigated.

Concentrations of 0.01 and 0.1 per cent oil of clove and 0.1 and 1.0 per cent of ground black pepper in tomato juice were selected for the present study.

Effect of the various spice concentrations on the thermal destruction of *B. thermoacidurans* is shown (Table 2). Concentrations of clove oil and pepper used were in excess of those normally employed in food products.

Effect of Sodium Benzoate and Ascorbic Acid: Although sodium benzoate may be used as a means of effecting limited preservation without need of heat sterilization, the effect of a 0.1 per cent concentration in tomato juice was investigated to determine whether or not efficiency of the heat treatments was increased upon addition of sodium benzoate.

Ascorbic acid is currently finding many applications in diverse branches of the food industry, where it functions as either an antioxidant or a means of fortification with more desirable levels of vitamin C. Inasmuch as a fortification level of 200 mg. of ascorbic acid per pint of juice has been recommended in some cases, the effect of this level on the thermal resistance of the test organism was studied.

Results obtained from rate of destruction curves and the TDT curves for these compounds are also given (Table 2).

DISCUSSION OF RESULTS

That incorporation of the various salt concentrations in tomato juice manifested itself by reductions in the thermal resistance of the standard inoculum of *B. thermoacidurans* is evident from results given (Table 2). Here it may be noticed that the effect of increasing the salt concentration was uniformly reflected by more rapid destruction rates and, therefore, shorter destruction times. In no case was there any evidence of any protective action afforded the test organism by any of the salt concentrations employed.

Whereas the influence of one per cent salt in reducing the destruction time was very slight, destruction time was reduced approximately 20 per cent by two per cent salt, 30 per cent by four per cent salt, and 40 per cent by eight per cent salt.

The reason for the action of salt in reducing the destruction times is not readily apparent as there is no obviously toxic entity in salt. Furthermore, as previously noted, there have been references in the literature that certain concentrations of salt actually protected certain organisms from the action of heat. It may well be that inasmuch as addition of increasing amounts of salt to tomato juice resulted in increased hydrogen ion concentrations, the lethal effect of salt was due, at least in part, to the lowered pH of the substrate.

Of all the food ingredients tested, the two sugars, sucrose and dextrose, were the only materials that enhanced the heat resistance of *B. thermoacidurans* in tomato juice. The increase in time necessary to obtain the desired endpoint of destruction progressed linearly with increase in percentages of sugars in the substrate.

Addition of the different acids in the same concentration resulted in differing pH values of the substrate according to the ionization constants of the individual acids. These differences in pH were further reflected in the time necessary for destruction of the inoculum—the lower the pH, the less time was required. Acetic acid with the lowest ionization constant was the least effective, on a percentage basis, of the three acids tested. Citric and lactic acids, whose ionization constants are quite similar, were about equally effective in reducing thermal destruction time of *B. thermoacidurans*.

As has been noted previously, when the pH of the tomato juice was adjusted to the same hydrogen ion concentration by means of lactic, acetic, and citric acids, differences in rates of destruction of the inoculum must be attributed to factors other than the hydrogen ion concentration. Levine and Fellers (1940) reported that the hydrogen ion activity is not solely responsible for the toxicity of acetic acid but that the undissociated acid molecule may be partially responsible for its lethal effect. This finding is supported by results shown (Table 2), where it is evident that at a pH of 3.8 acetic acid was definitely more effective than either lactic or citric acids.

Throughout these experiments there was little difference between effectiveness of lactic or citric acids at pH 3.8, although lactic acid was consistently somewhat more lethal than citric.

That the concentrations of spices used herein caused some reduction in destruction times of the test organism is shown (Table 2). For both oil of clove and black pepper, lowered destruction times were the result of increased concentrations of the spices, with the effectiveness of the 0.1 per cent oil of clove exceeding that of the 1.0 per cent ground black pepper.

Sodium benzoate at a level of 0.1 per cent in tomato juice proved to be slightly more effective in increasing rate of destruction of *B. thermoacidurans* than did ascorbic acid at a level of 200 mg. per pint of tomato juice. However, the difference in destruction times, when compared to those for the control tomato juice, were so slight as to be considered of no practical significance.

SUMMARY

The influence of various food ingredients in a tomato juice substrate on the thermal resistance of *B. thermoacidurans* was investigated.

The effect of adding salt in concentrations varying from one to eight per cent caused progressive decreases in the thermal destruction time.

Sucrose and dextrose were the only food materials that enhanced the heat resistance of the test organism. Incorporation of 50 per cent of either sucrose or dextrose in the substrate resulted in an increase of 200 to 300 per cent in destruction time over the control. Lower concentrations of these sugars gave correspondingly less protective effects.

Citric, acetic, and lactic acids, in concentrations of 0.5, one, and two per cent, proved to be effective in reducing the thermal death time of *B. ther-*

moacidurans. Based on the percentage of acid used, the relative efficiency of the acids in lowering the destruction time was: lactic > citric > acetic; while if based on the hydrogen ion concentration, the order would be: acetic > lactic > citric.

Neither 0.1 per cent sodium benzoate nor 0.2 per cent ascorbic acid (200 mg. per pint) in tomato juice resulted in significant changes in the thermal resistance of *B. thermoacidurans*.

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ACCEPTANCE OF SOLUBLE COFFEE

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A recent newspaper survey, Anon. (1949), indicates that in 12 cities across the nation the proportion of families purchasing soluble coffee ranges from approximately 10 to 42 per cent. It is generally assumed that the beverage derived from soluble coffee is distinctly different from that prepared from roasted, ground coffee beans so far as flavor quality is concerned. Prevalent opinions tend to agree that soluble coffee is inferior when compared with that made by approved methods, that it should be considered only as a food product having a new flavor, and finally that its popularity may be due only to its convenience. Although convenience is undoubtedly an important factor, no published information on the acceptability of soluble coffee in its various forms is available. Consequently, the study to be described was undertaken.

EXPERIMENTAL PROCEDURE

Experimental Design: The "paired-comparison" procedure described by Hening (1948) was used for the test. The coffee samples were presented to the judges in a series of pairs, each brand being paired in turn with every other brand in the series. Two pairs were presented each day, except during Series 3, when three pairs, or six samples, were presented at one time. Judges were required merely to decide their general preference between the two members of each pair. The significance of the preference was tested by means of the chi-square formula, Snedecor (1946). Four series of comparisons were made, e.g.: (1) between-brand comparisons of five pure soluble coffees, (2) between-brand comparisons of four soluble coffee products, (3) comparisons of three preferred brands of pure soluble coffee with three preferred brands of soluble coffee product, and (4) comparisons of three preferred brands of pure soluble coffee and three preferred brands of soluble coffee product with freshly-prepared dripolator coffee.

Judges: The judging group was made up of 77 (53 M, 24 F) unselected staff members and students. Regular attendance was not required; judges participated on whatever days and at whatever times were convenient. Daily attendance ranged from 26 to 46 and averaged 33 persons.

Tasting instructions and a brief statement of the object of the test were given orally. Judges tasted each pair of samples in any order, retasting and rinsing the mouth with water between tastes as desired, then recorded preferences on individual ballots.

Purchase, Preparation, and Storage of Test Materials: Five brands of pure soluble coffee (100 per cent coffee extract) and four brands of coffee product (50 per cent coffee extract plus 50 per cent added carbo-

hydrate in the form of dextrins, maltose, and dextrose) were purchased in local supermarkets.

Of the pure soluble coffees, Brands A, B, and C were fine, light powders, Brand D a golden-brown crystalline substance, and Brand E a dark-colored, slightly viscous liquid. Three of the coffee products (Brands F, G, and H) were fine, light powders. Brand I was a lumpy, grey-chocolate-colored powder which contained unsweetened, powdered milk. The powdered pure coffees were available in two-ounce containers, Brand E in eight-ounce bottles, and the coffee products in four-ounce containers, in each case the approximate equivalent of one pound of regular coffee. The nine brands ranged in price from 44 to 59 cents per equivalent pound. Supplies of each brand were pooled, mixed thoroughly, and placed in screw-top quart sealers. The jars were sealed and placed in a household refrigerator—approximately 4°C.(40°F.)—where they were stored for the 14 days of the test. The regular coffee, Brand X, was a popular, medium-priced brand, packed in one-pound vacuum cans retailing at 60 cents. This was purchased in a local supermarket and a fresh can opened every day that regular coffee appeared in the tests. A pasteurized, light (19 per cent butterfat) cream was delivered each morning by a local dairy. Ordinary cane sugar was used for sweetening.

Preparation and Serving of Samples: Soluble coffee samples were prepared individually. For expediency, the dry coffee was measured ahead of time, then, as each judge appeared, boiling water was poured on and the mixture stirred until dissolved. Proportions used had been worked out in preliminary trials so as to give mixtures of fairly comparable strength and color. The regular coffee was prepared in small, two-cup quantities in a china or enamel dripolator. When it could not be served immediately, it was held in a covered pot at a temperature below boiling. Fresh samples were prepared every 30 minutes, or more often when necessary.

Coffee was served black or with cream and/or sugar as requested. Where cream was added, a standard serving was used. Sugar was likewise added in a measured amount, the equivalent of one-half, one, or two teaspoonfuls per cup. All measurements were made by volume. The coffee was served in 150-ml. glass beakers in two and one-half-ounce (75-ml.) portions, the approximate equivalent of one-half teacup. Each person cooled his samples to his own taste, until they were of a temperature just comfortable for him to judge. Water was available for sipping between tastes.

RESULTS AND DISCUSSION

Table 1 shows the judges' preferences for the various brands of coffee presented in the four series of pairings.

On the basis of the preferences of Series 1, the brands of pure soluble coffee may be ranked in order of decreasing preference as B, C, A, D. The fifth brand, E, was tested only once (paired with D) and found to be not only completely lacking in coffee flavor but so unanimously unpalatable as a beverage that it was dropped from the test. When the chi-square formula is applied as a test of significance, it is found that at the $P = 0.05$ level Brand D is significantly less preferable than Brands A, B, and C, but that no other preferences are of significance. Thus, in so

far as the test indicates, Brands A, B, and C are about equally desirable, and Brand D is less preferable than any of these three. Consequently, Brands A, B, and C were selected for comparison with the coffee products and with regular coffee.

TABLE 1

Judges' Preferences for Pure Soluble Coffees, Soluble Coffee Products, and Regular Dripolator Coffee, Presented in Four Series of Pairings

Brand pairs	Judges' preferences	Significance of preferences [1]
Series 1		
A : B	18 : 20	.....
C : D	27 : 9	*
A : C	14 : 24	.....
B : D	28 : 9	*
B : C	22 : 18	.....
D : E	37 : 2	*
A : D	23 : 6	*
Series 2		
F : G	20 : 10	.....
F : H	13 : 20	.....
G : I	34 : 0	*
F : I	30 : 2	*
G : H	18 : 24	.....
H : I	37 : 1	*
Series 3		
A : F	10 : 16	.....
B : G	7 : 19	*
C : H	8 : 18	*
G : A	21 : 2	*
H : B	19 : 6	*
F : C	15 : 10	.....
A : H	9 : 17	.....
F : B	16 : 12	.....
C : G	5 : 21	*
Series 4		
B : X	20 : 17	.....
A : X	18 : 17	.....
X : G	8 : 19	*
F : X	15 : 13	.....
X : H	13 : 20	.....
X : C	17 : 17	.....

[1] *-Significant, P = 0.05. (Snedecor, 1946.)

On the basis of results of Series 2 the four soluble coffee products may be ranked in order of decreasing preference as H, F, G, I. At the P = 0.05 level Brand I is found to be significantly less preferable than Brands F, G, and H, whereas no other preferences are of significance. Since, in so far as the test would indicate, Brands F, G, and H are almost equally desirable and definitely more preferable than Brand I, these three were selected for further comparisons.

When each of the selected pure soluble coffees, A, B, and C, was compared in turn with each of the selected coffee products, F, G, and H, it

was found that the three coffee products were more preferable. In five of the nine choices involved in this series (G : A, G : B, G : C, H : B, and H : C) the preferences expressed are of significance, and in a sixth (H : A) the preference is close to significance. The three preferences involving Brand F (F : A, F : B, and F : C) are not sufficiently large to be of significance at the $P = 0.05$ level. The apparent inconsistency of these results with those of Series 1 and 2 is probably attributable to overlapping of the confidence limits, owing to the smaller number of judges taking part in Series 3. At any rate, it would appear that among the better brands of instant coffee, coffee products are in general preferred to pure soluble coffees.

When each of these six brands was paired in turn with Brand X, the regular coffee, it was found that one brand, C, was considered about equally as desirable as Brand X, whereas all others were considered somewhat preferable to Brand X. Only one of these preferences (G : X = 19 : 8) is significant at the $P = 0.05$ level. It is possible to state with confidence then that, on the basis of this test, Brand G is definitely preferred to regular coffee, whereas Brands A, B, C, F, and H are each about equally as desirable as regular coffee. Although preferences expressed in this series of comparisons are not generally of sufficient size to be significant, when they are ranged in order of decreasing magnitude the corresponding brands occur in the order of G, H, F = B, A, C, which is very comparable to the trend in Series 3.

It is preferable in an acceptability study that the products in question be tested under conditions as similar to those of normal consumption as possible. From this point of view coffee presents many special problems. It was felt desirable in this study to allow individual choice in tasting temperature and in addition of cream and sugar, although it is realized that in so doing, a lack of control is introduced. To treat the resulting data as an homologous unit in the statistical analysis is possible only if it be assumed that the variables are of negligible effect. In other words, the assumption is made that coffee preferences between different individuals and with one and the same individual are not influenced by use of cream and sugar. This means that the preference of an individual for, say, Coffee K compared with Coffee M is due solely to personal idiosyncrasy and is constant whether he compares the two brands black, or with cream, or with sugar. The truth or error of this basic assumption, upon which the statistical design of this experiment is predicated, influences the validity of the test.

The extreme variation among individuals in the concept of what constitutes quality in coffee presents a considerable problem in the choice of a standard or control. The variety of opinion voiced as to the desirability of the blend, roast, brew, color, strength, and freshness of the regular coffee, Brand X, indicated that for many people it was far from an ideal standard. Indeed, the possibility seems slight of serving any one coffee that would function as a satisfactory standard for a group of untrained judges in a test of this type.

SUMMARY AND CONCLUSIONS

A "paired-comparison" procedure was used to measure the relative acceptances of market brands of soluble coffee, and of soluble coffee and

regular coffee. Unselected staff members and students acted as judges.

Results indicated a limited "between-brand" preference in soluble coffee; among the better brands of both pure soluble coffee and soluble coffee product no brand preference was demonstrated. The more acceptable brands of soluble coffee product tend to be preferred to the more acceptable brands of pure soluble coffee. The better brands of both pure soluble coffee and soluble coffee product are about equally as desirable as regular dripolator coffee.

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